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SUBMISSION OF ENGLISH-LANGUAGE TRANSLATION OF PRIORITY DOCUMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

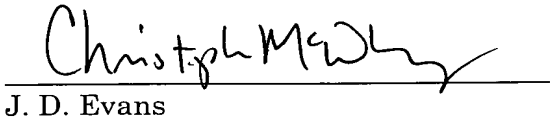
Sir:

Applicant submits herewith an English-language translation of the priority document, DE 101 28 541.8, filed on December 15, 2003, for the above-referenced application, together with a Declaration of the translator indicating that the translation is accurate.

The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Deposit Account No. 05-1323, Docket No.: 029310.52995US.

Respectfully submitted,

December 14, 2005



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DECLARATION

I, Janet Hope, BSc (Hons.), MIL., MITI., translator to Taylor and Meyer of 20 Kingsmead Road, London SW2 3JD, England, do solemnly and sincerely declare as follows:

1. That I am well acquainted with the English and German languages;
2. That the following is a true translation made by me into the English language of German Priority Text Application No. 101 28 541.8;
3. That all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true;
   and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardise the validity of the application or any patent issued thereon.

Signed this 19th day of September 2005

Stoke Goldington, Bucks, England.

Patent application by Grünenthal GmbH, D-52078 Aachen

(internal reference: G 3069)

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Screening method using BNPI and DNPI

The invention relates to a method for detecting pain-regulating substances using BNPI and/or DNPI and the use of compounds thereby identified, active compounds which bind to BNPI and/or DNPI, antibodies directed against BNPI and/or DNPI, of antisense nucleotides against BNPI and/or DNPI, or of BNPI and/or DNPI or part proteins thereof, and corresponding polynucleotides for medicaments for pain therapy and diagnostic agents.

Various medicaments are available for pain therapy, such as e.g. acetylsalicylic acid, paracetamol, dipyrone, tramadol, morphine and fentanyl; however, substances such as amitryptiline and ketamine are also employed for treatment of pain patients. In spite of increasingly refined therapy plans, however, often no permanent improvement can be achieved for the patients, especially in the case of chronic states of pain. The fact that with chronic pain permanent changes to the nerve cells involved occurs is also responsible, inter alia, for this.

Pain research in recent years has produced the fundamental finding that the development precisely of chronic states of pain are based on plastic changes to the nervous system, in particular in the nociceptive neurones of the posterior root ganglia and the neurones in the region of the dorsal horns of the spinal cord (as an overview see: Coderre et al. 1993; Zimmermann & Herdegen, 1996). The neuronal plasticity is accompanied by changes in the expression of certain genes and leads to a long-lasting change in the

phenotype of the neurones affected. The concept of neuronal plasticity has hitherto been applied above all to development, learning and regeneration processes, but recent findings from pain research show that this concept also intervenes in pathophysiological processes (Tölle, 1997).

The chronic development of pain has already been characterized relatively well at a phenomenological level in animal studies. Induction of chronic states of pain leads to the following changes:

- Increased sensitivity and reduced stimulus threshold of peripheral nociceptors
- Activation of so-called silent nociceptors
- Reorganization of receptive fields
- Increase in excitability in the spinal cord.

These plastic changes have been described both for the primary afferences which occur in the ganglia and for the subsequent neurones located in the spinal cord, and are also assumed to be supraspinal, e.g. in the thalamus. By analogy to the mechanisms described for learning and memory processes, it is to be assumed that a specific gene programme which comprises coordinated regulation of relevant genes, expression of which then contributes decisively to the pathophysiological manifestation of chronic pain, proceeds in the cells involved.

The starting point of the invention was therefore the identification of such pain-regulated which are modified in their expression under pain conditions and are therefore

probably involved, via their regulation connections, in the development and processing of, in particular, chronic pain.

A regulation has already been detected for a number of known genes in various pain models (see table 1), thus, for example, for neurotransmitters (substance P, CGRP), receptors (substance P receptor, μ-, κ-, δ-opiate receptors, NMDA receptor) and transcription factors (cJun, JunB, cFos or Krox24). The fact that the receptors mentioned are already used as molecular targets for the development of new analgesics (Dickenson, 1995) gives a clear indication that the identification of new pain-regulated genes is also of great interest for the development of analgesics, in particular for appropriate screening methods. The central idea here is to interrupt the development or persistence of pain, in particular of a chronic nature, by influencing the function of those proteins which are formed to an increased or decreased extent in states of pain.

Table 1: Regulation of known genes/gene products in pain animal models

Gene (product)	Reg	Tissue/cell	Model	Literature
(a) Neurotransmitters				
CGRP	⇑	SC dorsal horn	UV irradiation of the skin	Gillardon F et al. (1992) Ann NY Acad Sci657:493-96

Preprotachykinin & CGRP-mRNA	⇑	DRG	Monoarthritis	Donaldson LF et al. (1992) Mol Brain Res 16:143-49
Preprotachykinin-mRNA	⇑	SC dorsal horn	Formalin	Noguchi & Ruda (1992) J Neurosci 12:2563-72
Prodynorphin mRNA	⇑	Spinal cord	Exp. arthritis	Höllt et al. (1987) Neurosci Lett 96:247-52
Dynorphin prot.	⇑	Spinal cord	Formalin	Ruda et al. (1988) PNAS 85:622-26
Substance P	⇑	Nociceptors	Exp. arthritis	Levine JD et al. (1984) Science 226:547-49
(b) Neurotrophins				
BDNF mRNA & immune reactivity	⇑	DRG: trkA+ cells	i. th. NGF inj.	Michael GC et al. (1997) J Neurosci 17: 8476-90
(c) Receptors				
μ-, κ-, δ-binding	⇓⇑	SC dorsal horn	Monoarthritis	Besse D et al. (1992) Eur J Pharmacol 223:123-31
μ-Opiate receptor immune reactivity	⇑	DRG	Carrageenan ind. inflammation	Ji R-R et al. (1995) J Neurosci 15:8156-66
κ- & δ-opiate rec.-mRNA	⇓	DRG	Carrageenan ind. inflammation	Ji R-R et al. (1995) J Neurosci 15:8156-66
κ- & μ-opiate receptor-mRNA	⇑	SC dorsal horn	Monoarthritis	Maekawa K et al. (1995) Pain 64:365-71

CCK_B-rec. mRNA	⇑	DRG	Axotomy	Zhang X et al. (1993) Neuroscience 57:227-233
NMDA-R1-mRNA	⇓	SC dorsal horn laminae I & II	CFA-induced inflammation	Kus L et al. (1995) Neuroscience 68:159-65
(d) Enzymes				
NADPH-diaphorase activity	⇑	SC dorsal horn	Ischiaticus transection	Fiallos-Estrada et al. ('93) Neurosci. Lett 150:(169-73)
NADPH-diaphorase	⇑	Spinal cord	Formalin	Solodkin et al. 1992 Neurosci 51:495-99
NO synthetase mRNA	⇑	DRG	Axotomy	Verge VMK et al. (1992) PNAS 89:11617-62
NO synthetase protein	⇑	SC dorsal horn	Formalin	Herdegen et al. (1994) Mol Brain Res 22:245-58
NO synthetase immune reactivity	⇑	DRG	Ischiaticus transection	Fiallos-Estrada et al. ('93) Neurosci Lett 150:169-73
(e) Signal cascades				
rap1A, rap1B, H-ras mRNA	⇑	Spinal cord	Formalin	Urayama O et al. (1997) Mol Brain Res 45:331-34
PKC-binding	⇑	SC dorsal horn	CFA-induced monoarthritis	Tölle TR et al (82) J Neurol 242(S2):135

(f) Transcription f.				
cFOS	⇑	Spinal cord	Noxic stimulation	Hunt SP et al. (1987) Nature 328:632-34
cJun, JunB, cFOS Krox24	⇑	SC dorsal horn	Formalin	Herdegen T et al. (1994) Mol Brain Res 22:245-48

SC, spinal cord; DRG, dorsal root ganglia; CFA, complete Freund's adjuvant; NGF, nerve growth factor

Following from this, the primary object of the invention was to develop a screening method for identification of substances relevant in pain, in particular pain-regulating substances. The invention therefore relates to a method for detecting pain-regulating substances with the following method steps:

(a) incubation under suitable conditions of a substance to be tested with the protein BNPI or DNPI and/or a protein according to one of figures 1b), 1d), 1f), 2b), or 2d) and/or a protein which is similar to one of these abovementioned proteins to the extent of at least 90% and/or a protein for which a polynucleotide according to one of figures 1a), 1c), 1e), 2a), or 2c) or a polynucleotide which is similar thereto to the extent of at least 90% codes, and/or a protein which is coded by a nucleic acid which bind under stringent conditions to a polynucleotide according to one of figures 1a), 1c), 1e), 2a),

or 2c) or antisense polynucleotides thereof, or a part protein of one of the abovementioned proteins which is at least 10, preferably at least 15, in particular at least 20 amino acids long and/or a cell and/or a preparation from such a cell which has synthesized at least one of the abovementioned proteins and part proteins,

(b) measurement of the binding of the test substance to the protein or part protein synthesized by the cell or measurement of at least one of the functional parameters modified by the binding of the test substance to the protein or part protein.

This novel screening method is based on the fact that a potential pain activity of a substance can be detected via its interaction with a pain-regulated protein structure, BNPI or DNPI or related structures.

The term pain-regulating here relates to a potential regulating influence on the physiological pain event, in particular to an analgesic action. The term substance includes any compound suitable as a medicament active compound, in particular, that is to say, low molecular weight active compounds, but also others, such as nucleic acids, fats, sugars, peptides or proteins, such as antibodies.

Incubation under suitable conditions is to be understood here as meaning that the substance to be investigated can react with the cell or the corresponding preparation in an aqueous medium a defined time before the measurement. The

aqueous medium can be temperature-controlled here, for example between 4°C and 40°C, preferably at room temperature or at 37°C. The incubation time can be varied between a few seconds and several hours, depending on the interaction of the substance with the part protein or protein. However, times of between 1 min and 60 min are preferred. The aqueous medium can comprise suitable salts and/or buffer systems, so that, for example, a pH of between 6 and 8, preferably pH 7.0 - 7.5, prevails in the medium during the incubation. Suitable substances, such as coenzymes, nutrients etc., can furthermore be added to the medium. The suitable conditions can easily be specified by the expert as a function of the interaction to be investigated of the substance with the part protein or protein on the basis of his experience, the literature or a few simple preliminary experiments in order to obtain the clearest possible measurement value in the method.

A cell which has synthesized a particular part protein or protein is a cell which has already expressed this part protein or protein endogenously or one which has been modified by genetic engineering such that it expresses this part protein or protein and accordingly contains the part protein or protein before the start of the method according to the invention. The cells can be cells of possibly immortalized cell lines or can be native cells originating from tissues and isolated from these, the cell union usually being broken down. The preparation from these cells comprises, in particular, homogenates from the cells, the cytosol, a membrane fraction of the cells with membrane fragments, a suspension of isolated cell organelles etc.

The proteins and part proteins listed here have been identified in the context of this invention as regulated by pain or distributed in a pain-relevant manner by inducing pain in an animal and, after an appropriate period of time, comparing the expression pattern of the animal with that of a control animal without pain-inducing measures by sections in the spinal cord. Those found here as modified in expression are BNPI and, in particular in respect of pain-relevant distribution, DNPI.

The species from which these proteins originate is irrelevant for the functioning of the method, but it is preferable to use the human, mouse or rat variants. BNPI and DNPI are known in respect of the coding DNA sequence and the amino acid sequence and are also described in their general function. BNPI, the "brain Na+ dependent inorganic phosphate cotransporter", is described in WO 96/34288 and DNPI, the "differentiation-associated Na+ dependent inorganic phosphate cotransporter", has been described by Aihara et al. (2000) in J. Neurochem. 74, 2622-2625. However, neither of these transporters has hitherto been connected with pain and, in particular, pain regulation in the prior art. Since the identification of the proteins took place here via a modification of the expression or via the expression distribution in an in vivo pain model, for future medicaments using these proteins the screening method according to the invention derived therefrom has the considerable advantage not only of being built up on theoretical considerations but presumably of having a strong in vivo relevance. Since with this method the interaction of substances with proteins and peptides not hitherto used in the pain sector is rendered possible as a

standard for detecting pain-regulating substances, pain-relevant substances which would not have emerged in the methods known hitherto in the prior art using other peptides or proteins are now possibly to be detected with this method. This is also a considerable advantage of the new method according to the invention.

The standard via which the method allows the detection of interesting substances is either the binding to the protein or part protein, which can be detected e.g. by displacement of a known ligand or the extent of the substance bound, or the modification of a functional parameter due to the interaction of the substance with the part protein or protein. This interaction can lie, in particular, in a regulation, inhibition and/or activation of receptors, ion channels and/or enzymes, and modified functional parameters can be, for example, the gene expression, the ionic medium, the pH or the membrane potential, or the modification of the enzyme activity or the concentration of the 2nd messenger.

To explain the invention, in addition to the explanations given for terms in the general text, further definitions are given below in order to clarify how certain terms used in the claims in particular are to be understood and interpreted in the context of this invention.

- Substance: By this is meant a chemical compound. In the narrower sense, these are compounds which can potentially display an action in the body, low molecular weight active compounds, nucleic acids, fats,

sugars, peptides or proteins, low molecular weight active compounds in particular here.

- Pain-regulating: In the context of the invention, pain-regulating means that the substance directly or indirectly influences the perception of pain, in particular has a natural analgesic action.

- Pain: In the context of the invention, pain means in particular a pain sensation, more precisely acute, chronic, neuropathic and inflammatory pain, including migraine, and in particular the pain belongs to the following types:

  chronic pain, in particular musculoskeletal pain; neuropathic pain, in particular allodynic pain, mechanical hyperalgesia or diabetic neuropathy; visceral pain, cerebral pain, peripheral pain or inflammation-related pain, in particular peripheral inflammation pain; and migraine, cluster headache or pain with trigeminus neuralgia.

- Incubation: Incubation is to be understood as meaning the procedure in which a biological object for investigation, for example a cell or a protein, is introduced into and left in a temperature-controlled medium, such as in an incubating cabinet or on a water-bath. Suitable conditions here means incubation under physiological conditions (e.g. 37°C pH 7.2) or under conditions under which an optimum measurement in the method is possible.

- Cell: The cell is a self-regulating, open system which is in a flow equilibrium with its environment by permanent exchange of matter and has its own metabolism and ability to multiply. The cell can be cultured separately or can be part of a tissue, in particular from an organ, and can exist there individually or also in the cell union.

- Preparation from a cell: This is understood as meaning preparations which are prepared by means of chemical, biological, mechanical or physical methods with a change in the cell structure, for example membrane fragments, isolated cell compartments, isolated cytosol, or homogenate obtained from tissue.

- Peptide: Compound of amino acids linked to chains via peptide bonds. An oligopeptide consists of between 2 and 9 amino acids and a polypeptide of between 10 and 100 amino acids.

- Protein: Compound of more than 100 amino acids linked to chains via peptide bonds, under certain circumstances with a defined spatial structure.

- Part protein: Compound of more than 10 amino acids linked to chains via peptide bonds, under certain circumstances with a defined spatial structure, but cut out or selected from a defined protein. A part protein can be a peptide.

- PIM1-kinase: PIM3-kinase: A proto-oncogene and a serine-threonine kinase.

- Polynucleotide: The underlying nucleotide is in principle a base unit of nucleic acids which consists of nuclein base, pentose and phosphoric acid. This corresponds to a high molecular weight polynucleotide of several nucleotides linked to one another via phosphoric acid-pentose esterification. However, this invention also includes modified polynucleotides, which indeed retain the base sequence but have a modified backbone instead of phosphoric acid-pentose.

- Similar to the extent of at least 90 (95, 97)%: This is to be understood as meaning that in their coding region the polynucleotides referred to are at least 90% (95%, 97%) identical to the reference (figure etc.) with respect to the base sequence, and in their primary structure, the sequence of amino acids, the peptides and proteins referred to are identical to the extent of at least 90% (95%, 97%) to the reference.

- Gene: The term gene describes a genome section with a defined nucleotide sequence which contains the information for synthesis of an m- or pre-mRNA or another RNA (e.g. tRNA, rRNA, snRNA etc). It consists of coding and non-coding sections.

- Gene fragment: Nucleic acid section which comprises a part region of a gene in its base sequence.

- Binding to the peptide, part protein or protein: Interaction between substance and peptide, part protein or protein which leads to fixing.

- Functional parameters: This is understood as meaning measurement parameters of an experiment which correlate with the function of a protein (ion channel, receptor, enzyme).

- Manipulated by genetic engineering: Manipulation of cells, tissues or organisms such that genetic material is introduced here.

- Expressed endogenously: Expression of a protein which shows a cell line under suitable culture conditions without this corresponding protein having been prompted to expression by manipulation by genetic engineering.

- G protein: Internationally conventional abbreviation for a guanosine triphosphate (GTP)-binding protein which is activated as a signal protein by receptors coupled to G protein.

- Reporter gene: General term for genes of which the gene products can be detected easily with the aid of simple biochemical methods or histochemical methods, such as e.g. luciferase, alkaline phosphatase or green fluorescent protein (GFP).

- (Recombinant) DNA construct: General term for any type of DNA molecules which have formed by in vitro linking of DNA molecules.

- Cloning vector: General term for nucleic acid molecules which serve as carriers of foreign genes or parts of these genes during cloning.

- Expression vector: Term for specially constructed cloning vectors which, after introduction into a suitable host cell, allow transcription and translation of the foreign gene cloned into the vector.

- LTR sequence: Abbreviation for long terminal repeat. General term for longer sequence regions which are to be found at both ends of a linear genome. Such sequence regions occur e.g. in the genomes of retroviruses and at the ends of eukaryotic transposons.

- Poly A tail: The adenyl radicals attached at the 3' end of messenger RNA by polyadenylation (approx. 20-250).

- Promoter sequence: Term for a DNA sequence region from where the transcription of a gene, i.e. the synthesis of the mRNA, is controlled.

- ORI sequence: Abbreviation for origin of replication. The ORI sequence allows a DNA molecule to multiply as an autonomous unit in the cell.

- Enhancer sequence: Term for relatively short, genetic elements, which in some case occur as repetitions and which as a rule enhance the expression of some genes to a varying degree.

- Transcription factor: Term for a protein which influences the transcription of a gene via binding to specific DNA sequences.

- Culturing: Keeping cells or tissue under suitable culture conditions.

- Conditions which allow expression: By this is understood the choice and use of culture conditions which allow expression of the protein of interest, which include change in temperature, change of medium, addition of inducing substances, omission of inhibiting substances.

- Incubation time: Duration of time for which cells or tissue are incubated, i.e. exposed to a defined temperature.

- Selection pressure: Applications of culture conditions which provide cells which have a particular gene product, the so-called selection marker, with a growth advantage.

- Amphibia cell: Cell from an animal of the Amphibia class.

- Bacteria cell: Cell which is to be assigned to the superkingdom of Eubacteria or Archaebacteria or originates from it.

- Yeast cell: Cell which is to be assigned to the order of the Endomycetales or originates from it.

- Insect cell: Cell which is to be assigned to the order of the Hexapoda or originates from it.

- Native mammalian cell: Cell originating from a mammal which corresponds in its relevant features to the cell present in the organism.

- Immortalized mammalian cell: Cell which has acquired, by the culture conditions applied or manipulation by genetic engineering, the property of dividing in the culture beyond the usual conventional frequency of division (approx. 100).

- Labelled: Rendered accessible to a detection reaction by appropriate modification or derivatization. For example radioactively, fluorescently or luminescently.

- Ligand: Substance which binds to a molecule present in the body or a cell, specifically a receptor.

- Displacement: Complete or partial removal of a ligand from its binding site.

- Bound activity: Biochemically or physically recorded measurement value which correlates with the amount of ligand bound to a receptor.

- Regulation: The inhibition or activation of a process which has taken place as part of a regulating process.

- Inhibition: Inhibition/reduction of a process as a special case of regulation.

- Activation: Intensification of a process as a special case of regulation.

- Receptors: In the broadest sense, all the molecules present in the pro- or eukaryotic organism which can bind to an active compound. In the narrower sense, membrane-bound proteins or complexes of several proteins which cause a change in the cell by binding an active compound.

- Ion channels: Membrane-bound proteins or complexes of several proteins by which cations or anions can pass through the membrane.

- Enzymes: Term for proteins or complexes of an activating non-protein component with a protein which has catalytic properties.

- Gene expression (express/expressible): The translation of the genetic information of a gene into RNA (RNA expression) or into protein (protein expression).

- Ionic medium: Ion concentration of one or more ions in a particular compartment.

- Membrane potential: Potential difference over a membrane on the basis of an excess of cations on one side and anions on the other side of the membrane.

- Change in enzyme activity: Inhibition or induction of the catalytic activity of an enzyme.

- 2nd messenger: Small molecule which, as a response to an extracellular signal, either is formed in the cytosol or migrates into the cytosol and thereby helps to transmit information to the inside of the cell, such as, for example, cAMP, IP_3.

- (Gene) probe: Term for any type of nucleic acids with the aid of which a gene sought or a particular DNA sequence can be detected. By derivatization of the gene probe (e.g. biotin, magnetic beads, digoxinin), DNA molecules can furthermore be drawn out of a mixture. Cloned genes, gene fragments, chemically synthesized oligonucleotides and also RNA, which is usually radioactively labelled, are used as probes.

- DNA: International term for deoxyribonucleic acid.

- Genomic DNA: General term for the DNA originating from the cell nucleus of a cell in eukaryotic organisms.

- cDNA: Abbreviation for complementary DNA. Term for the single- or double-stranded DNA copy of an RNA molecule.
- cDNA bank/library: Term for a collection of arbitrarily cloned cDNA fragments which, taken together, represent the entirety of all the RNA synthesized by a cell or a tissue.

- cDNA clone: Term for a population of genetically uniform cells which are derived from a single cell such

that this cell contains an artificially introduced cDNA fragment.

- Hybridization: Formation, effected by base pairing, of a double-stranded nucleic acid molecule from two separate single strands.

- Stringent conditions: Conditions under which only perfectly base-paired nucleic acid strands are formed and remain stable.

- Isolate: To discover and separate off a molecule sought from a mixture.

- DNA sequencing: Determination of the sequence of from bases in a DNA molecule.

- Nucleic acid sequence: Term for the primary structure of a DNA molecule, i.e. the sequence of the individual bases from which a DNA is composed.

- Gene-specific oligonucleotide primer: Oligonucleic acids, that is to say nucleic acid fragments 10-40 bases long, which, in their base composition, allow a stringent hybridization to the gene sought or the cDNA sought.

- Determination of oligonucleotide primers: The manual or computer-assisted search for oligonucleotides for a given DNA sequence which are of optimum suitability for a hybridization and/or a polymerase chain reaction.

- PCR: Abbreviation for polymerase chain reaction. In vitro process for selective concentration of nucleic acid regions of defined length and defined sequence from a mixture of nucleic acid molecules.

- DNA template: Nucleic acid molecule or a mixture of nucleic acid molecules from which a DNA section is multiplied with the aid of the PCR (see above).

- RNA: Internationally common abbreviation for ribonucleic acids.

- mRNA: Internationally common abbreviation for messenger ribonucleic acids which are involved in transfer of the genetic information from the nucleus into the cell and contain information for the synthesis of a polypeptide or a protein.

- Antisense polynucleotide: A molecule comprising several natural or modified nucleic acids, the base sequence of which is complementary to the base sequence of a part region of an RNA which occurs in nature.

- PNA: Internationally common abbreviation for peptidic nucleic acids. Peptidically linked amino acids form a chain here, the amino acids carrying as a side chain a base which is capable of hybridization with DNA or RNA.

- Sequence: Sequence of nucleotides or amino acids. In the specific context of this invention, this means the nucleic acid sequence.

- Ribozyme: Term for a catalytically active ribonucleic acid (e.g. ligase, endonuclease, polymerase, exonuclease).

- DNA enzyme: Term for a DNA molecule which contains catalytic activity (e.g. ligase, endonuclease, polymerase, exonuclease).

- Catalytic RNA/DNA: General term for ribozymes or DNA enzymes (see above).

- Adenovirus: Cytopathogenic virus which occurs in vertebrates.

- Adeno-associated virus (AAV): Belongs to the family of Parvoviruses. For effective multiplication of AAV, co-infection of the host cells with helper viruses (e.g. herpes, vaccinia or adeno-viruses) is necessary. The property of AAV of integrating into the host genome in a stable manner makes it of particular interest as a transduction vector for mammalian cells.

- Herpes virus: Viral pathogen of herpes infection
- Post-translational modification: Modification to proteins or polypeptides carried out after translation, which includes e.g. phosphorylation, glycosylation, amidation, acetylation or proteolysis.

- Glycosylate: Term for the appending of individual sugar molecules or whole sugar chains on to proteins.

- Phosphorylate: Term for the appending of one or more phosphate radicals on to a protein, preferably on to the OH groups of the amino acids serine, threonine or tyrosine.

- Amidate: The term for conversion of a carboxyl function into an amide function, e.g. on the carboxy-terminal amino acid radical of a peptide or protein.

- Provided with a membrane anchor: Post-translational modification of a protein or of another organic molecule such that, by appending a hydrophobic molecule, suitably a fatty acid or a derivative thereof, it is anchored to the lipid double-layer membrane of cells.

- Cleave: In this specific case cleavage of a peptide or protein into several sub-sequences.

- Shorten: Shortening of a molecule consisting of several individual parts by one or more parts.

- Antibodies: Proteins, called immunoglobulins, which are soluble or bound to cell membranes and have a specific binding site for antigens.

- Monoclonal antibodies: These are antibodies which have an extremely high selectivity and are directed against a single antigenic determinant of an antigen.

- Polyclonal antibodies: Mixture of antibodies directed against several determinants of an antigen.

- Transgenic: Genetically modified.

- Non-human mammal: The entirety of mammals (class of Mammalia) with the exception of the human species.

- Germ cell: Cell with a haploid genome which, by fusion with a second germ cell, renders possible the formation of a new organism.

- Somatic cell: Diploid cell as a constituent of an organism.

- Chromosomal introduction: Intervention in the nucleotide sequence at the chromosomal level.

- Genome: General description of the entirety of all the genes in an organism.

- Ancestor of the animal: An animal (the ancestor) which is related in a direct line with another animal (the descendant) in a natural or artificial manner by passing on at its genetic material.

- Expressible: A nucleic acid molecule is expressible if it contains the information for synthesis of a protein or polypeptide and is provided with appropriate regulatory sequences which allow synthesis of this protein or polypeptide in vitro or in vivo. If these prerequisites no longer exist, for example by intervention into the coding sequence, the nucleic acid molecule is no longer expressible.

- Rodent: Animal from the order of the Rodentia, e.g. rat or mouse.

- Substance identifiable as pain-regulating: Substance which, when introduced into a living organism, causes a change in behaviour which the expert calls pain-inhibiting (antinociceptive, antihyperalgesic or antiallodynic). In the case of the screening method, this relates to the fact that, during screening, the substance significantly, for example by 100%, exceeds the binding or interaction of the average substances tested due to stronger binding or inducement of a modification in a functional parameter.

- Compound: Another name for a molecule consisting of several atoms, in this case a molecule identified by the method according to the invention.

- Active compound: A compound which, when used on an organism, causes a change in this organism. In particular, by this is understood molecules synthesized by organic chemistry which have a healing action on the organism. Here in particular molecules which bind to the proteins and peptides according to the invention.

- Low molecular weight: Molecule with a molecule weight of < 2 kDa.

- Medicament: A substance corresponding to the definition in article 1 §2 of the Act on Circulation of Medical Preparations.

- Diagnostic agent: Compound or method which can be used to diagnose a disease.

- Treatment of pain: Method with the aim of alleviating or eliminating pain or inhibiting the expected occurrence of pain (pre-emptive analgesia).

- Chronic pain: A pain sensation of longer-lasting duration, often characterized in that it increases the pain sensitivity of the body beyond the point in time and location of the initial stimulus.

- Gene therapy: Gene therapy is understood as meaning all methods which have the aim of causal treatment of genetic diseases by suitable modifications to the genome.

- In vivo gene therapy: Introduction of genetic material into the living organism with the aim of gene therapy. A distinction can be made between somatic and germ path intervention, which takes place in one instance on diploid cells and in the other instance on haploid cells.

- In vitro gene therapy: Introduction of genetic material into cells outside the human body with the aim of subsequently using these again for gene therapy by introduction into the human body.

- Diagnostics: Methods for identifying a disease.

- Investigation of activity: Investigation with the aim of investigating the activity of a compound after acting on a living organism.

In a preferred embodiment of the method, the cell is manipulated by genetic engineering before step (a). In this procedure, genetic material is introduced into the cell, in particular one or more polynucleotide sequences. In a variant of this embodiment which is furthermore preferred, the manipulation by genetic engineering allows the measurement of at least one of the functional parameters modified by the test substance. In this embodiment, prerequisites under which the modification of a functional parameter can be measured at all or in an improved manner are created by manipulation by genetic engineering. It is particularly preferable here for a form of a G protein which is not expressed endogenously in the cell to be expressed or a reporter gene to be introduced by the manipulation by genetic engineering. This is to be understood, in particular, as meaning the introduction into the cell, by genetic engineering, of a G protein (GTP-binding protein) which is not present endogenously or is not expressed physiologically, for example the introduction of a chimaeric G protein which allows a modification of the signal path or of a promiscuous G protein which binds very readily. The introduction of a reporter gene in turn allows the measurement of an (extracellularly triggered) induced expression of the gene product.

In a further preferred embodiment, the cell is manipulated by genetic engineering such that the cell contains at least one polynucleotide according to one of figures 1a), 1c),

1e), 2a), or 2c) or a polynucleotide which is similar thereto to the extent of at least 90%. The achievement of this can be, for example, that a part protein or protein which is not expressed endogenously in the cell or preparation used in the method is synthesized by the cell. It is particularly preferable here for the polynucleotide to be contained in a recombinant DNA construct. A (recombinant) DNA construct is understood as meaning a DNA molecule prepared in vitro.

If the cell is manipulated by genetic engineering before step a) in the method, it is preferable for the cell to be cultured, after the manipulation by genetic engineering and before step a), under conditions which allow an expression, optionally under selection pressure. Culturing is understood as meaning keeping cells or tissue under conditions which ensure survival of the cells or their subsequent generation. The conditions should be chosen here such that an expression of the material inserted by the manipulation by genetic engineering is rendered possible. For this, the pH, oxygen content and temperature should be kept at the physiological values and sufficient nutrients and necessary cofactors should be added. The selection pressure allows only the cells in which the manipulation by genetic engineering was at least partly successful to be cultured further. This includes, for example, introduction of an antibiotic resistance via the DNA construct.

It is particularly preferable in the method according to the invention for the cell used to be an amphibia cell, bacterial cell, yeast cell, insect cell or an immortalized

or native mammalian cell. Examples for amphibia cells are Xenopus oocytes, for bacteria cells E. coli cells, for yeast cells those also Saccharomyces cerevisiae, for insect cells Sf9 cells, for immortalized mammalian cells HeLa cells and for native mammalian cells the CHO (Chinese hamster ovary) cell.

In a preferred measurement method for determination of the binding of the substance to part protein or protein in the method according to the invention, the measurement of the binding is carried out via the displacement of a known labelled ligand of the part protein or protein and/or via the activity bound thereto from a labelled test substance. A ligand here is a molecule which binds to the protein or part protein with a high specificity and is displaced from the binding site by a substance to be tested which also binds. Labelling is to be understood as meaning an artificial modification to the molecule which facilitates detection. Examples are radioactive, fluorescent or luminescent labelling.

In another preferred measurement method for determination of the modification of the functional parameter induced by the binding of the substance to the part protein or protein in the method according to the invention, measurement of at least one of the functional parameters modified by the test substance is carried out via measurement of the regulation, inhibition and/or activation of receptors, ion channels and/or enzymes, in particular via measurement of the modification in gene expression, the ionic medium, the pH or the membrane potential, via the modification in the enzyme activity or the concentration of the 2nd messenger.

This includes on the one hand measurement of the action of the substance directly via influencing of receptors, ion channels and/or enzymes, and on the other hand, as examples which are preferably to be measured, measurement of parameters which are modified, such as gene expression, ionic medium, pH, membrane potential, enzyme activity or concentration of the 2nd messenger. Ionic medium is understood here as meaning, in particular, the concentration of one or more ions in a cell compartment, in particular the cytosol, membrane potential is understood here as meaning the charge difference between two sides of a biomembrane, and 2nd messenger is understood here as meaning messenger substances of the intracellular signal path, such as e.g. cyclic AMP (cAMP), inosotol triphosphate (IP3) or diacylglycerol (DAG).

This method includes the use of part proteins and in particular proteins with a known sequence and function, without a function in pain being known for these in the prior art.

A method according to the invention which is furthermore preferred is that wherein the pain regulated by the substance to be detected is chosen from:

chronic pain, in particular musculoskeletal pain; neuropathic pain, in particular allodynic pain, mechanical hyperalgesia or diabetic neuropathy; visceral pain, cerebral pain, peripheral pain or inflammation-related pain, in particular peripheral inflammation pain; and migraine, cluster headache or pain with trigeminus neuralgia.

The invention also provides a compound which is identifiable as a pain-regulating substance by a method according to the invention. Compound here relates in particular to low molecular weight active compounds, and also to peptides, proteins and nucleic acids. Identifiable here means that the compound has the feature that in the screening method according to the invention it binds significantly more strongly in respect of the binding, preferably twice as strongly, as the average of the substances to be tested or deviates significantly from the average of the substances to be tested in respect of the modification of the functional parameters. It is particularly preferable for the compound according to the invention to be a low molecular weight compound.

The invention also relates to the use of

a. a polynucleotide, preferably a DNA or RNA, which codes for BNPI or DNPI or a polynucleotide, preferably a DNA or RNA, which corresponds to the extent of at least 90%, preferably 95%, in particular to the extent of at least 97%, to one of the nucleotide sequences shown in figures 1a), 1c), 1e), 2a), 2c) or 2e),

b. a polynucleotide, in particular an antisense polynucleotide or a PNA, preferably a DNA enzyme or ribozyme, a ribozyme or other DNA enzyme or a catalytic RNA or DNA, which has a nucleotide sequence which is capable of binding specifically to one of the polynucleotides listed under point a),

c. a vector containing a polynucleotide according to one of points a) or b), in particular an expression vector and/or in particular derived from a virus, for example the adenovirus, adeno-associated virus or herpes virus, and/or in particular containing at least one LTR, poly A, promoter and/or ORI sequence,

d. BNPI or DNPI and/or a protein according to one of figures 1b), 1d), 1f), 2b) or 2d) and/or a protein which is similar to one of these abovementioned proteins to the extent of at least 90% and/or a protein for which a polynucleotide according to one of figures 1a), 1c), 1e), 2a) or 2c) or a polynucleotide which is similar thereto to the extent of at least 90% codes, and/or a protein which is coded by a nucleic acid which binds under stringent conditions to a polynucleotide according to one of figures 1a), 1c), 1e), 2a) or 2c) or antisense polynucleotides thereof or a part protein of one of the abovementioned proteins which is at least 10, preferably at least 15, in particular at least 20 amino acids long, wherein the protein or part protein has been optionally post-translationally modified, in particular glycosylated, phosphorylated, amidated, methylated, acetylated, ADP-ribosylated, hydroxylated, provided with a membrane anchor, cleaved or shortened,

e. an antibody, preferably a monoclonal or polyclonal antibody, against one of the proteins or part proteins according to point d),

f. a cell, preferably an amphibia cell, bacteria cell, yeast cell, insect cell or an immortalized or native mammalian cell, containing a polynucleotide

according to one of points a) or b), a vector according to point c), a protein or part protein according to point d) or an antibody according to point e)

g. a compound according to one of claims 12 or 13 and/or

h. an active compound, preferably a low molecular weight active compound, which binds to a protein or part protein according to point a),

for the preparation of a medicament for treatment of pain.

The use for treatment of chronic, in particular neuropathic or inflammation-related pain is particularly preferred.

The invention also provides the use of

a. a polynucleotide, preferably a DNA or RNA, which codes for BNPI or DNPI or a polynucleotide, preferably a DNA or RNA, which corresponds to the extent of at least 90%, preferably 95%, in particular to the extent of at least 97%, to one of the nucleotide sequences shown in figures 1a), 1c), 1e), 2a), 2c) or 2e),

b. a polynucleotide, in particular an antisense polynucleotide or a PNA, preferably a DNA enzyme or ribozyme, a ribozyme or other DNA enzyme or a catalytic RNA or DNA, which has a nucleotide sequence which is capable of binding specifically to one of the polynucleotides listed under point a),

c. a vector containing a polynucleotide according to one of points a) or b), in particular an expression vector and/or in particular derived from a virus,

for example the adenovirus, adeno-associated virus or herpes virus, and/or in particular containing at least one LTR, poly A, promoter and/or ORI sequence,

f. a cell, preferably an amphibia cell, bacteria cell, yeast cell, insect cell or an immortalized or native mammalian cell, containing a polynucleotide according to one of points a) or b) or a vector according to point c)

for the preparation of a medicament for use in gene therapy. It is particularly preferable here for the therapy to be in vivo or in vitro gene therapy. Gene therapy is understood as meaning a therapy form in which an effector gene, usually a protein, is expressed by introduction of nucleic acids into cells. A distinction is made in principle between in vivo and in vitro methods. In the case of in vitro methods, cells are removed from the organism and transfected ex vivo with vectors, in order to be subsequently introduced again into the same or into another organism. In the case of in vivo gene therapy, vectors, for example for combating tumours, are administered systemically (e.g. via the blood stream) or directly into the target tissue (e.g. into a tumour). It is furthermore preferable for the medicament furthermore to be a medicament for treatment of pain.

In the use in gene therapy, the use of a polynucleotide which is an antisense polynucleotide or PNA, or which is part of a ribozyme or other DNA enzyme or of a catalytic RNA or DNA is also preferred.

The invention also furthermore provides the use of

a. a polynucleotide, preferably a DNA or RNA, which codes for BNPI or DNPI or a polynucleotide, preferably a DNA or RNA, which corresponds to the extent of at least 90%, preferably 95%, in particular to the extent of at least 97%, to one of the nucleotide sequences shown in figures 1a), 1c), 1e), 2a), 2c) or 2e),

b. a polynucleotide, in particular an antisense polynucleotide or a PNA, preferably a DNA enzyme or ribozyme, a ribozyme or other DNA enzyme or a catalytic RNA or DNA, which has a nucleotide sequence which is capable of binding specifically to one of the polynucleotides listed under point a),

c. a vector containing a polynucleotide according to one of points a) or b), in particular an expression vector and/or in particular derived from a virus, for example the adenovirus, adeno-associated virus or herpes virus, and/or in particular containing at least one LTR, poly A, promoter and/or ORI sequence,

d. BNPI or DNPI and/or a protein according to one of figures 1b), 1d), 1f), 2b) or 2d) and/or a protein which is similar to one of these abovementioned proteins to the extent of at least 90% and/or a protein for which a polynucleotide according to one of figures 1a), 1c), 1e), 2a) or 2c) or a polynucleotide which is similar thereto to the extent of at least 90% codes, and/or a protein which is coded by a nucleic acid which binds under stringent conditions to a polynucleotide according to one of figures 1a), 1c), 1e), 2a) or 2c) or antisense polynucleotides thereof or a part protein

of one of the abovementioned proteins which is at least 10, preferably at least 15, in particular at least 20 amino acids long, wherein the protein or part protein has been optionally post-translationally modified, in particular glycosylated, phosphorylated, amidated, methylated, acetylated, ADP-ribosylated, hydroxylated, provided with a membrane anchor, cleaved or shortened,

e. an antibody, preferably a monoclonal or polyclonal antibody, against one of the proteins or part proteins according to point d),

f. a cell, preferably an amphibia cell, bacteria cell, yeast cell, insect cell or an immortalized or native mammalian cell, containing a polynucleotide according to one of points a) or b), a vector according to point c), a protein or part protein according to point d) or an antibody according to point e)

g. a compound according to one of claims 12 or 13 and/or

h. an active compound, preferably a low molecular weight active compound, which binds to a protein or part protein according to point a),

for the preparation of a diagnostic agent for diagnosis of a pain state. Diagnostics is understood here as meaning the analysis of symptoms assigned to a disease syndrome, and investigations of activity are understood as meaning investigations of the activity of substances to be tested, in particular their medicinal activity.

The invention furthermore also provides a process for the preparation of a peptide or protein according to the invention, in which a cell according to the invention which contains a polynucleotide according to the invention and/or a vector according to the invention is cultured and the peptide or protein is optionally isolated.

The invention also provides the use of

a. a polynucleotide, preferably a DNA or RNA, which codes for BNPI or DNPI or a polynucleotide, preferably a DNA or RNA, which corresponds to the extent of at least 90%, preferably 95%, in particular to the extent of at least 97%, to one of the nucleotide sequences shown in figures 1a), 1c), 1e), 2a), 2c) or 2e),

b. a polynucleotide, in particular an antisense polynucleotide or a PNA, preferably a DNA enzyme or ribozyme, a ribozyme or other DNA enzyme or a catalytic RNA or DNA, which has a nucleotide sequence which is capable of binding specifically to one of the polynucleotides listed under point a),

c. a vector containing a polynucleotide according to one of points a) or b), in particular an expression vector and/or in particular derived from a virus, for example the adenovirus, adeno-associated virus or herpes virus, and/or in particular containing at least one LTR, poly A, promoter and/or ORI sequence,

d. BNPI or DNPI and/or a protein according to one of figures 1b), 1d), 1f), 2b) or 2d) and/or a protein which is similar to one of these abovementioned proteins to the extent of at least 90% and/or a

protein for which a polynucleotide according to one of figures 1a), 1c), 1e), 2a) or 2c) or a polynucleotide which is similar thereto to the extent of at least 90% codes, and/or a protein which is coded by a nucleic acid which binds under stringent conditions to a polynucleotide according to one of figures 1a), 1c), 1e), 2a) or 2c) or antisense polynucleotides thereof or a part protein of one of the abovementioned proteins which is at least 10, preferably at least 15, in particular at least 20 amino acids long, wherein the protein or part protein has been optionally post-translationally modified, in particular glycosylated, phosphorylated, amidated, methylated, acetylated, ADP-ribosylated, hydroxylated, provided with a membrane anchor, cleaved or shortened,

e. an antibody, preferably a monoclonal or polyclonal antibody, against one of the proteins or part proteins according to point d),

f. a cell, preferably an amphibia cell, bacteria cell, yeast cell, insect cell or an immortalized or native mammalian cell, containing a polynucleotide according to one of points a) or b), a vector according to point c), a protein or part protein according to point d) or an antibody according to point e)

in a method for detecting pain-regulating substances.

Generally, it is preferable for all the abovementioned uses according to the invention for the pain to be chosen from

chronic pain, in particular musculoskeletal pain; neuropathic pain, in particular allodynic pain, mechanical hyperalgesia or diabetic neuropathy; visceral pain, cerebral pain, peripheral pain or inflammation-related pain, in particular peripheral inflammation pain; and migraine, cluster headache or pain with trigeminus neuralgia.

The polynucleotide used according to the invention also includes the gene fragments described themselves, as well as a polynucleotide which corresponds either completely or at least in parts to the coding sequence of the gene corresponding to the fragment. This also means polynucleotides which have at least 90%, preferably 95%, in particular at least 97% agreement in the base sequence with the coding sequence of the polynucleotides shown or the coding sequence of the gene. It is furthermore preferable for the polynucleotide to be RNA or single- or double-stranded DNA, in particular mRNA or cDNA. It is also preferable for the polynucleotide to be an antisense polynucleotide or PNA which has a sequence which is capable of binding specifically to a polynucleotide according to the invention. PNA is understood here as meaning "peptidic nucleic acid", which indeed carries the base pairs but the backbone of which is bound peptidically. An antisense polynucleotide shows the complementary base sequence to at least a part of a base nucleic acid. It is also preferable for the polynucleotide to be part of a ribozyme or other DNA enzyme or of a catalytic RNA or DNA. Ribozyme is to be understood as meaning a catalytically active ribonucleic acid, and DNA enzyme is to be understood as meaning a

corresponding deoxyribonucleic acid, that is to say catalytic RNA or DNA.

The vector used according to the invention is understood as meaning a nucleic acid molecule which serves to contain or transfer foreign genes in manipulation by genetic engineering. It is particularly preferable here for the vector to be an expression vector. It therefore serves for expression of the foreign gene contained therein, the polynucleotide. Such a vector which is derived from a virus, for example the adenovirus, adeno-associated virus or herpes virus, and/or it contains at least one LTR, poly A, promoter and/or ORI sequence is furthermore preferred. An LTR is a "long terminal repeat", a section at the end, for example in viruses. Poly A sequence is a tail more than 20 adenosine radicals long. A promoter sequence is the control region for the transcription.

For a protein used or a part protein derived therefrom, it is preferable for this to have been post-translationally modified, for it to have been, in particular, glycosylated, phosphorylated, amidated, methylated, acetylated, ADP-ribosylated, hydroxylated, provided with a membrane anchor, cleaved or shortened. Post-translational modifications can be found, for example, in Voet/Voet, Biochemistry, 1st Edition, 1990, p. 935-938.

For a use according to the invention, it is particularly preferable here for the polynucleotide (optionally according to point a) and/or point b)) to be an RNA or a single- or double-stranded DNA, in particular, mRNA or cDNA.

For a use according to the invention, it is particularly preferable here for the polynucleotide (optionally according to point b)) to be part of a ribozyme or other DNA enzyme or of a catalytic RNA or DNA.

For a use according to the invention, it is particularly preferable here for the vector (optionally according to point c)) to be an expression vector.

For a use according to the invention, it is furthermore particularly preferable here for the vector (optionally according to point c)) to be derived from a virus, for example the adenovirus, adeno-associated virus or herpes virus, and/or to contain at least one LTR, poly A, promoter and/or ORI sequence.

For a use according to the invention (not gene therapy), it is particularly preferable here for the protein or part protein (optionally according to point d)) to have been post-translationally modified, in particular to have been glycosylated, phosphorylated, amidated, methylated, acetylated, ADP-ribosylated, hydroxylated, provided with a membrane anchor, cleaved or shortened.

For a use according to the invention (not gene therapy), it is particularly preferable here for the antibody (optionally according to point e)) to be a monoclonal or polyclonal antibody.

For a use according to the invention, it is particularly preferable here for the cell (optionally according to point

f)) to be an amphibia cell, bacteria cell, yeast cell, insect cell or an immortalized or native mammalian cell.

For a use according to the invention, it is particularly preferable here for the compound (optionally according to point g)) to be a low molecular weight compound.

For a use according to the invention, it is particularly preferable here for the active compound mentioned, according to point h), to be a low molecular weight active compound.

The invention also provides a process for pain treatment of a non-human mammal or human which or who requires treatment of pain, in particular chronic pain, by administration of a medicament according to the invention, in particular one comprising a substance according to the invention and/or an active compound which binds BNPI and/or DNPI.

The administration can take place, for example, in the form of a medicament as described above.

Overall, an important basis of the invention is the identification of pain-regulated genes and gene fragments. The screening method is based on this. However, the use for diagnosis or therapy is also available, as already stated. Appropriate possible uses and further embodiment examples are explained in the following.

1. Therapy of chronic pain

mRNA expression of kinases was investigated by in situ hybridization in spinal cord tissue. In the spinal cord,

the primary sensory neurones project to subsequent central nervous neurones, these being, in addition to supraspinal processes, the central switching site for nociceptive information. Numerous experiments have shown that the development of chronic states of pain is based on plastic changes in the nervous system (as an overview see Corderre et al., 1993; Zimmermann and Herdegen, 1996). In the neurones of the dorsal root ganglia and spinal cord in particular, plastic changes which are accompanied by regulation of pain-relevant genes have been described. Gene regulation in the spinal cord has thus been described for a number of neurotransmitter receptors which are of importance for pain therapy (see table 1). On this basis, the cDNA sequences found which are regulated under pain could be used for therapy (gene therapy, antisense, ribozymes) and diagnosis of chronic states of pain.

1.1 Antisense strategies

Constructs which are derived from the nucleic acid sequence of the complete cDNA or from part regions and which can reduce the mRNA or protein concentration are established here. These can be e.g. antisense oligonucleotides (DNA or RNA), which have an increased stability towards nucleases, possibly using modified nucleotide units (e.g. O-allyl-ribose). Furthermore, the use of ribozymes, which, as enzymatically active RNA molecules, catalyse a specific cleavage of the RNA, is conceivable. In addition, vectors which express the sequences according to the invention or part regions of these nucleotide sequences under control of a suitable promoter and are therefore suitable for an in vivo or ex vivo therapy could also be employed. Antisense constructs which, under exchange of the phosphate backbone

of nucleotide sequences (e.g. PNAs, i.e. peptide nucleic acid) or by using non-traditional bases, such as inosines, queosines or wybutosines, as well as acetyl-, methyl-, thio- and similarly modified forms of adenine, cytidine, guanosine u, thymidine and uridine, cannot be degraded or can be degraded to a relatively low degree by endogenous nucleases are additionally also possible.

1.2. Antagonists/agonists or inhibitors/activators of the gene products according to the invention used in the screening method.

This includes substances which, by binding to the gene product, modify the function thereof. These can be:

1.2.1. Organic chemical molecules which are found in the context of an active compound screening using the gene products of the cDNA according to the invention as binding partners.

1.2.2. Antibodies, whether polyclonal, chimaeric, single-chain, F_{ab} fragments or fragments from phage banks, which preferably specifically influence the function as neutralizing antibodies via binding to the gene products.

1.2.3. Aptamers, i.e. nucleic acids or nucleic acid derivatives with protein-binding properties. These also include so-called mirror-mers, which are mirror-image and therefore stable oligonucleotides obtained by mirror evolution and can bind a target molecule with a high affinity and high specificity (Klußmann et al., 1996).

1.3. Gene therapy

The sequences described can be employed for therapy of neurological diseases, in particular chronic states of pain, by using them, after cloning into suitable vectors

(e.g. adenovirus vectors or adeno-associated virus vectors), for in vivo or ex vivo therapy in order there e.g. to counteract an over-expression or under-expression of the endogenous gene product, to correct the sequence of the defective gene product (e.g. by trans-splicing with the exogenous construct) or to provide a functional gene product.

2. Diagnosis

Polynucleotide sequences (oligonucleotides, antisense DNA & RNA molecules, PNAs) which are derived from the nucleotide sequences used in the screening method etc. could be employed for diagnosis of states or diseases associated with an expression of these gene sequences. Examples of these states or diseases include neurological diseases, including chronic pain or neuropathic pain (caused e.g. by diabetes, cancer or AIDS), or neurodegenerative diseases, such as Alzheimer's, Parkinson's, Huntington's Chorea, Jacob-Creutzfeld's, amyotrophic lateral sclerosis and dementias. The nucleotide sequences can serve in diverse ways (northern blot, southern blot, FISH analysis, PRINS analysis, PCR) either for identification of the gene product or deviating diagnostically relevant gene products or for quantification of the gene product. In addition to nucleic acid diagnostics, antibodies or aptamers against the protein coded by the nucleic acids according to the invention can also be employed for diagnostics (e.g. by means of ELISA, RIA, immunocytochemical or immunohistochemical methods) in order to identify the protein or deviating forms and to quantify the protein.

In respect of gene diagnostics, nucleic acid probes derived from the nucleotide sequences according to the invention could be employed for determination of the gene locus (e.g. by FISH, FACS, artificial chromosomes, such as YACs, BACs or P1 constructs).

The following examples and figures are intended to illustrate the invention without limiting it thereto.

Figures and examples

Figures:

Fig. 1a) cDNA sequence of BNPI, human; AN: NM_020309

Fig. 1b) Amino acid sequence of PIM1-kinase, human; AN: NM_020309

Fig. 1c) cDNA sequence of BNPI, human; no.: AAT42064 from WO96/34288

Fig. 1d) Amino acid sequence of BNPI, human; no.: AAT42064 from WO96/34288

Fig. 1e) cDNA sequence of BNPI, rat; AN: U07609

Fig. 1f) Amino acid sequence of BNPI, rat; AN: U07609

Fig. 2a) cDNA sequence of DNPI, human; AN: AB032435

Fig. 2b) Amino acid sequence of DNPI, human; AN: AB032435

Fig. 2c) cDNA sequence of DNPI, rat; AN: AF271235

Fig. 2d) Amino acid sequence of DNPI, rat; AN: AF271235

Fig. 3) Separation of radioactively labelled RFDD-PCR fragments in a 6% denaturing PAA gel (see example 1)

Fig. 4) Upwards regulation of DNPI and BNPI protein expression in primary sensory rat DRG neurones and fibres after collagen-induced arthritis. (see example 2)

Fig. 5) Differential expression of DNPI and BNPI in synapses of pain conduction and motor areas of the lumbar spinal cord of the rat (see example 3a)

Fig. 6) Differential expression of DNPI and BNPI in synapses of the dorsal horn pain conduction areas of the lumbar spinal cord of the rat (see example 3b)

Fig. 7) Differential expression of DNPI and BNPI in synapses of pain conduction of the sacral spinal cord of the rat (see example 3c)

Fig. 8) Differential expression of DNPI and BNPI in synapses of medullo-cervicospinal pain conduction of the trigeminal nerve of the rat (see example 3d)

Fig. 9) Differential expression of DNPI and BNPI in pain-relevant brain regions of the rat (see example 3e)

Fig. 10) Differential expression of DNPI and BNPI in pain-relevant brain regions of the rat (see example 3f)

Fig. 11) Differential expression of DNPI and BNPI in pain-relevant brain regions of the rat (see example 3g)

Fig. 12) Differential expression of DNPI and BNPI in pain-relevant brain regions of the rat (see example 3h)

Examples:

Example 1: Identification of pain-regulated genes by means of RFDD-PCR

A) Procedure

The following procedure was chosen:
CFA-induced arthritis in the rat in which complete Freund's adjuvant is injected into the tail root was chosen as the starting point for isolation of pain-regulated genes. The target tissue in which the pain-regulated expression of the genes according to the invention was detected was the dorsal root ganglia of the fifth lumbar segment. Four methods are available for isolation of differentially regulated genes:

- cDNA-RDA (cDNA-representational difference analysis; Hubank & Schatz, 1994)

- DDRT-PCR (differential display RT-PCR; Liang & Pardee 1992, Bauer et al., 1994), There have since been improved modifications of this, such as the so-called "restriction fragment differential display PCR" (RFDD-PCR), which allows a more reproducible reaction by additional restriction fragmenting of the cDNA in combination with an optimized PCR amplification and furthermore detects fragments to an increased extent in the coding region (Ivanova et al., 1995).

- Subtractive hybridization (Watson & Margulies, 1993)

• SAGE (serial analysis of gene expression, Velculescu et al., 1995).

A comparative evaluation of the methods mentioned led to selection of RFDD-PCR, since in contrast to subtractive hybridization and SAGE, this method is capable of detecting both upwards- and downwards-regulated genes and also rare transcripts and moreover provides an abundance of results within short periods of time.

B) MATERIAL AND METHODS

Isolation and characterization of pain-regulated cDNA sequences

Animal model: CFA-induced polyarthritis

Adjuvant arthritis (AA) is an induced form of (sub)chronic arthritis. It is induced by immunizing rats with a suspension of mycobacteria in oil. The disease thereby induced is an autoimmune arthritis which is mediated by T cells and which - since, however, no defined autoantigen is employed during the induction - corresponds to an arthritis which occurs spontaneously in humans. AA is often used for investigations of immunological aspects of rheumatoid arthritis. Furthermore, the model is used for testing antiinflammatory and analgesic substances. AA is a fairly aggressive form of arthritis. The inflammation process of AA is indeed self-healing, but severe joint changes nevertheless persist. The severity of the disease can be quantified by drawing up an arthritis index. All four paws are inspected here for redness, swelling and

deformation of the joints. The course of the disease can furthermore be characterized more closely via determination of the body weight and of the paw swelling by means of plethysmography and by histological examinations of the joints.

The arthritis is induced by intracutaneous injection of CFA (100 µl of the 5 mg/ml stock solution) into the tail root (dorsal). The severity of the arthritis is determined with the aid of a scoring index by daily observation of the animals for mobility, reddening of the skin and swellings of the tarsal and carpal joint. The onset of visible inflammations of the tarsal or carpal joint starts on about day 10 after immunization. The severity of the disease increases over a period of 10-14 days, reaches an optimum which is maintained for about 6-7 days, to then subside again. If rats were immunized only with IFA, no arthritis was induced.

Removal of tissue. The animal are decapitated and the dorsal root ganglia are removed after lubalectomy and immediately frozen in liquid nitrogen.

RNA isolation. The total RNA was isolated from the tissue samples with the Trizol Kit (Life Technologies) in accordance with the manufacturer's instructions. The RNA was quantified by UV spectrometry (extinction at 260 nm) and checked for integrity by denaturing gel electrophoresis in a formaldehyde-agarose gel (Sambrook et al., 1989).

DNase digestion. Before use in the DDRT-PCR, any traces of genomic DNA are removed by DNase digestion. In this, in each case 6 µg RNA were incubated in a total

volume of 100 µl in 1X First-Strand Buffer (Life Techn.) and 10 units of RNase-free DNaseI (Boehringer Mannheim) for 15 minutes at 37°c. After phenol/chloroform extraction, the RNA was precipitated by addition of 1/10 vol. sodium acetate pH 5.2 and 2.5 vol. ethanol, dissolved in DEPC water, quantified by UV spectrometry and characterized by renewed formaldehyde-agarose gel electrophoresis.

Reverse transcription. In each case 1 µg of DNaseI-digested RNA were subjected to reverse transcription with the aid of the displayProfile Kit (Display Systems Biotech, Vista, USA) in accordance with the manufacturer's instructions and double-stranded cDNA was produced. After purification of the cDNA by phenol/chloroform extraction and ethanol precipitation, the efficiency of the cDNA synthesis was detected by gel electrophoresis in a 1.5% agarose gel.

Taq1 restriction digestion. In each case 10 µl of the double-stranded cDNA were digested with the restriction enzyme Taq1. This was also carried out with the aid of the displayProfile Kit (Display Systems Biotech, Vista, USA) in accordance with the manufacturer's instructions. Starting from this batch, adapters are ligated to the digested cDNA.

^{33}P end labelling reaction. For subsequent detection of the fragments, one of the two primers (so-called O-extension primers) was radioactively labelled by an end labelling reaction with T4 polynucleotide kinase and [γ^{33}P]ATP. This was also carried out with the aid of the displayProfile Kit (Display Systems Biotech, Vista, USA) in accordance with the manufacturer's instructions.

PCR amplification of the cDNAs. After ligation, in each case 0.2 µl of the cDNA are amplified in parallel reaction batches with the labelled o-extension primer and one of the 64 Eu primers and the reaction batches are separated by electrophoresis in a 6% Tris-taurine-EDTA-polyacrylamide gel. The gel was then dried for one hour at 80°C and exposed overnight on a BASIII detection screen (Fuji). The STORM-Phosphorus Imager (Molecular Dynamics) using the ImageQuant software was used for the evaluation. The autoradiography data were printed on film in the same scale, which was then used for cutting out the fragments.

Reamplification of the DDRT-PCR fragments. Differentially regulated PCR bands were cut out of the gel with a scalpel and eluted from the piece of gel by boiling for 15 minutes in 50 µl Tris-EDTA buffer, were reamplified by PCR with the aid of the displayProfile Kit (Display Systems Biotech, Vista, USA) in accordance with the manufacturer's instructions. The temperature profile corresponded to the original PCR reaction (see above). 10 µl sample buffer (0.25% bromophenol blue, 0.25% xylenecyanol FF, 30% glycerol) then added to the PCR batches, separation carried out by gel electrophoresis in a 3% TAE-agarose gel with 10 µg/ml ethidium bromide and PCR products of the expected size cut out of the gel.

Cloning into TA cloning vectors. The fragments cut out were purified with the Qiaquick Gel Extraction Kit (Qiagen) in accordance with the manufacturer's instructions, concentrated to dryness and taken up in 5 µl doubly dist. water. They were then ligated into the pCRII-TOPO vector by means of the TOPO TA Cloning Kit

(Invitrogen) in accordance with the manufacturer's instructions and transformed in TOP10F'-E. coli cells. The transformation batch was plated out on LB-agar plates with 100 µg/ml ampicillin, which had been treated beforehand with 50 µl 2% X-Gal (Sigma) and 50 µl isopropyl thiogalactoside (Sigma). The white bacteria clones obtained after incubation for 15 hours at 37°C were transferred into 5 ml LB liquid medium with 100 µg/ml ampicillin (100 µg/ml) and incubated overnight at 37°C, while shaking. Plasmid DNA was isolated from these cultures using the Qiagen Spin Miniprep Kit (Qiagen) in accordance with the manufacturer's instructions and in each case 5 µl of the plasmid DNA were characterized by EcoRI restriction digestion and subsequent TAE-agarose gel electrophoresis.

Sequence analysis. In this, in each case 500 ng of the plasmid DNA were sequenced with the T7-PCR primer using the Dye Terminator Cycle Sequencing Kit (Perkin-Elmer) in accordance with the manufacturer's instructions and the reactions were analysed by means of the automatic sequencer ABI 370 (Applied Biosystems Inc.). The DNA sequences were compared with the gene libraries using bioSCOUT software (LION, Heidelberg).

C) Result

A corresponding autoradiogram is shown in figure 3. The autoradiogram shows the separation of PCR fragments which have formed by amplification of various cDNAs. The cDNAs were synthesized by reverse transcription from total RNA from L5 spinal ganglia. The total RNA was isolated from

control animals (-) and CFA-treated animals (+). The fragment ab50-24 which, as shown by means of the RFDD method, has an upwards regulation is identified with an arrow. The fragment ab50-24 shows a highly significant homology to the cDNA sequence AC no. AAT42064 of hBNPI (see fig. 1c). It is therefore demonstrated that BNPI is expressed more intensively under the conditions of a CFA treatment.

Example 2
Identification of pain-regulated genes via immunocytochemical staining

The following procedure was chosen:

The so-called CIA model(collagen-induced arthritis) in the rat, in which colagen is injected in order to induce arthritis in the rat was chosen as the starting point for isolation of pain-regulated genes.

The procedure corresponded to the method described by Persson S., Schäfer MK-H., Nohr D., Ekström G., Post C., Nyberg F. and Weihe E. (1994), Neuroscience 63; 313-326 and Nohr D., Schäfer MK-H., Romeo H., Persson S., Nyberg F. Post C. and Weihe E. (1999), Neuroscience 93; 759-773, the disclosure of this article expressly being made part of the disclosure of the invention submitted here.

Polyclonal rabbit antisera against the recombinant DNPI or BNPI fusion protein were used for the immunohistochemical staining. It was found in figure 4 that the intensity of the DNPI and BNPI immunostaining in

the lumbar dorsal root ganglion of the arthritic rat (B and D/CIA) increased compared with the control animals (A and C/CTLR). The increase both in the cell bodies and the fibre staining in B compared with A and in C compared with D is to be noted.

Example 3

Differential consideration of the expression between DNPI and BNPI via immunocytochemical staining

Polyclonal rabbit antisera against the recombinant DNPI and BNPI fusion protein were used for the immunohistochemical staining. Generally, sections of various regions of the CNS were prepared and the expression of DNPI was compared with that of BNPI.

Example 3a on figure 5)

The differential distribution of the immune reactivity of BNPI and DNPI in the lumbar spinal cord of the rat is to be seen. The adjacent deparafinized sections A- to D are stained as follows:

A = anti-DNPI;
B = anti-DNPI preadsorbed with DNPI fusion protein;
C = anti-BNPI;
D = anti-BNPI preadsorbed with BNPI fusion protein;

The DNPI (A) and BNPI (C) immunodyestuffs were completely preadsorbable with homologous recombinant BNPI (D) and BNPI (B) fusion protein, which proves the specificity of the immune reaction.

The mutually exclusive distribution pattern of DNPI and BNPI immunostaining in the outer and deep dorsal horn is remarkable. (A;C). Pointwise immunostaining of DNPI is in the synaptic endings of the outer dorsal horn (lamina 1 and substantia gelatinosaa) (arrow in A), while BNPI immune reactivity is completely absent (arrows in B). Accumulation of intense positive pointwise BNPI immunostaining exists in the deeper dorsal horn, while DNPI staining is relatively low. DNPI is present in the lateral spinal nucleus (LSN in A), while BNPI is completely absent (LSN in C). DNPI is abundant in the lamina X around the central canal, while BNPI is rare. BNPI immunostaining is weak in the lateral ventral horn and slight or absent in the medial ventral horn. Pointwise DNPI staining is abundant through the entire ventral horn, but somewhat less in the lateral horn compared with the medial ventral horn. There is a weak BNPI and DNPI staining in some cell bodies of the ventral horn motoneurone, but this was not preadsorbed by the homologous transport fusion proteins and was therefore classified as non-specific.

Example 3b on figure 6)

The differential distribution of the immune reactivity of BNPI and DNPI in the left lateral superficial dorsal lumbar spinal cord of the rat is to be seen. A and B, stained in each case for BNPI (A) and DNPI (B), show many pointwise stains for DNPI, which are concentrated in the lamina I and substantia gelatinosa, where BNPI is almost completely absent. Dense complexes of DNPI-positive points are furthermore to be seen in the lateral spinal nucleus, where

BNPI is almost completely absent. Fine DNPI-positive points are also to be found in the deeper dorsal horn, although in a lower density.

Example 3c on figure 7)

The differential distribution of the immune reactivity of BNPI and DNPI in the sacral spinal cord of the rat is to be seen. The adjacent sections A and B, stained in each case for BNPI (A) and DNPI (B), show mutual exclusion zones of pointwise DNPI and BNPI immunostaining in the dorsal horn. DNPI is present in the entire grey matter and is concentrated in the very outer layers of the dorsal horn, where a narrow band forms at the boundary to the white matter. DNPI is abundant in the lateral spinal nucleus and in the lamina X, and also in the lamina V/VI and in the entire ventral horn. BNPI is abundant in the deep dorsal horn and rare in the ventral horn.

Example 3d on figure 8)

The differential distribution of the immune reactivity of BNPI and DNPI in the lower medulla oblongate at the transition to the cervical spinal cord can be seen. The adjacent sections A and B, in each case stained for BNPI (A) and DNPI (B), show a preferred accumulation of the BNPI staining in the medial part of the spinal trigeminal nucleus and in the middle and lower part of the dorsal medulla. only a very weak staining is to be seen with BNPI in the ventral medulla. DNPI is abundant in the grey matter of the medulla. DNPI staining overlaps with the BNPI staining in the inner spinal nucleus V. It is to be

noted that BNPI is also to be found in the upper spinal trigeminal nucleus, which is the same as the spinal substantia geloatinosa. DNPI staining is weaker in areas in which BNPI is present, weaker than in areas where BNPI is low or absent. A few BNPI points are to be seen in the ventral grey motor area.

Example 3e on figure 9)

Complementarily differential distribution of DNPI and BNPI immune reactivity in 2 consecutive sections of the rat brain in pain-relevant brain regions, such as the sensory parietal cortex; cingular cortex, thalamus, corpus amygdaloideum and also hypothalamus. DNPI is concentrated in the cortex in the granular sensory layers, in particular in lamina IV; BNPI is abundant in the cortex but weaker in lamina IV than in other laminae. In the cingular cortex (C vs D as a magnification), the distribution of DNPI and BNPI is complementarily mutually exclusive or reciprocal in the density of the particular synapses. DNPI clearly predominates over BNPI in the thalamus, BNPI is sparse in the hypothalamus, DNPI abundant. Abundant BNPI predominates in the hypocampus over sparse DNPI with mutually complementary distribution.

Thalamus = Th,

Amygdala = Amyg.

Hippocampus = Hip,

Cingular cortex = Cg,

Hypothalamus = Hy,

Parietal cortex = PC.

Example 3f on figure 10)

Complementarily differential distribution of DNPI and BNPI immune reactivity in pain-relevant brain regions, such as the cingular cortex (Cg) and tectum and dorsal periaqueductal grey. DNPI dominance in the tectum and dorsal grey. Consecutive sections of a rat brain through the upper mesencephalon.

Example 3g on figure 11)

Complementarily differential distribution of DNPI and BNPI immune reactivity in pain-relevant brain regions, such as the tectum (T) and periaqueductal grey (PAG). DNPI dominance in the tectum and dorsal grey. Differential distribution of DNPI and BNPI in the corpus geniculatum mediale (cgm) of the auditory path is to be noted. Consecutive sections of a rat brain through the upper mesencephalon; colliculus superior plane.

Example 3h on figure 12)

Abundance of DNPI over BNPI in the habenulae (Hb). DNPI is present in the entire habenular complex (low magnification, upper figure; high magnification, middle fig.). BNPI is only in the medial habenular core (mHb lower fig., consecutive section to the middle figure).

Analysis of example 3 generally:

The differential distribution of BNPI and DNPI in synapses of the primary afferent, spinal trigeminal and supraspinal nociceptive system is strong evidence of a selective influencability of nociceptive functions by selective modulation of the DNPI- or BNPI-mediated glutamate transport. The distribution of BNPI in the deep dorsal horn is an indication of a preferential role of BNPI in glutamate-driven neuropathic pain.

The preferential distribution of DNPI in lamina 1 and the substantia gelatinosa of the spinal and trigeminal nociceptive system suggests a primary and preferential role of DNPI in inflammation pain. Since DNPI synapses also lie in the deeper dorsal horn, DNPI is also a candidate in the case of neuropathic pain.

BNPI is a preferential candidate for allodynia and mechanical hyperalgesia with inflammation pain. Glutamate-mediated Aβ input converging on spinal nociceptive projection neurones could be a substantial mechanical for chronic deep musculoskeletal pain, a main problem of chronic pain.

The presence in visceral sacral afferences points to an indication in visceral pain.

Trigeminal afference: migraine, cluster headache, trigeminus neuralgia.

Example 4:

Procedure for the screening method with measurement of the binding via the displacement of a radioactively labelled ligand

A nucleic acid section which codes for BNPI is cloned in an expression vector which allows a constitutive expression (e.g. CMV promoter) or an inducible expression in eukaryotic cells. The DNA is introduced with suitable transfection processes, e.g. with Lipofectamin (Roche Diagnostics), into eukaryotic cells (e.g. CHO cells, HEK293 cells or NIH-3T3 cells). The cells are cultured in the presence of a selection reagent (e.g. zeocin, hygromycin or neomycin) such that only the cells which have taken up the DNA construct and, during longer-lasting selection, also incorporated it into the genome survive.

Starting from these cells, membrane fractions which contain BNPI in a large amount and can be used for a binding assay are obtained. This assay consists of 1.) the membranes containing BNPI, 2.) a radioactively labelled ligand, 3.) a binding buffer (e.g. 50 mM HEPES pH 7.4, 1 mM EDTA) and the ligand to be investigated for binding. After incubation of the abovementioned reaction mixtures (for e.g. 30-60 min) at a suitable temperature (usually room temperature), the non-bound radioactive ligand molecules are filtered off. The remaining amount of bound ligand is measured, after addition of a scintillation cocktail, in a β-counter (e.g. Trilux, Wallac). If the test substance shows binding to the BMPI, this is detected as a reduced radioactive incorporation. This method is suitably miniaturized such that it can be carried out in (96-, 384- or 1,536-well) microtitre plates in order to carry out this method by

means of a robot in the so-called high troughput screening (HTS) method.

Example 5:

Procedure for the screening method according to the invention with BNPI and measurement of the functional parameters modified by binding of the substance

A nucleic acid section which codes for BNPI is cloned in an expression vector which allows an inducible expression in prokaryotes, such as e.g. E. coli. The nucleic acid section is modified here such that it is expressed as a fusion protein with an additional N- or C-terminal amino acid sequence. This sequence should allow, with a non-modified function of the BNPI, a purification via a specific method, e.g. glutathione S-transferase fragment, which allows isolation from the protein mixture via binding to glutathione. After transfection of the bacteria, induction of the gene (e.g. with IPTG in the case of the lac promoter) and breaking down of the bacteria, the fusion proteins are purified and employed in an in vitro kinase experiment. In this, 5 µg protein are at 30°C for 30 minutes in 50 µl kinase buffer (20 mM PIPES, pH 7.0, 5 mM $MnCl_2$, 7 mM β-mercaptoethanol, 0.4 mM spermine, 10 mM rATP) supplemented with 10 µCi [γ^{32}P] ATP. Purified histone H1 protein (Sigma) or bacterially expressed GST-NFATc1 fusion protein are added as substrates. After the incubation time, the non-incorporated [γ-^{32}P] ATP is filtered off and the amount of ^{32}phosphate incorporated is determined by β-scintillation (Trilux, Wallac). In an experiment for discovering new BNPI inhibitors, the test substances are co-incubated in this batch and a decrease in the ^{32}P

incorporation is used as an indicator for an inhibitor. This method is suitably miniaturized such that it can be carried out in (96-, 384- or 1,536-well) microtitre plates in order to carry out this method by means of a robot in the so-called high troughput screening (HTS) method.

Example 6:
Procedure for the screening method according to the invention with DNPI and measurement of the functional parameters modified by binding of the substance

The method is carried out as described in example 5, with the exception that instead of a nucleic acid section which codes for BNPI, a nucleic acid section which codes for DNPI was employed.

Example 7:
Example of a medicament for pain treatment comprising a compound according to the invention - tablet formulation

Tablets can be prepared by direct pressing of mixtures of the compound according to the invention with corresponding auxiliary substances or by pressing granules containing the compound (with optionally further auxiliary substances). The granules can be prepared here either by moist granulation with e.g. aqueous granulating liquids and subsequent drying of these granules or by dry granulation, e.g. via compacting

- Direct pressing

e.g. per tablet:	25 mg	compound according to the invention
	271 mg	LudipressTM (granules for direct tablet making from lactose monohydrate, povidone K30 and crospovidone)
	4 mg	magnesium stearate
	300 mg	total

Prepare a homogeneous mixture of the active compound with the auxiliary substances and press this on a tablet press to give tablets with a Ø of 10 mm.

- Dry granulation

e.g. per tablet:	25 mg	compound according to the invention
	166 mg	microcrystalline cellulose
	80 mg	hydroxypropylcellulose with a low degree of substitution (I-HPC LH 11™)
	5 mg	highly disperse silicon dioxide
	4 mg	magnesium stearate
	280 mg	total

Prepare a homogeneous mixture of the compound with the microcrystalline cellulose and the I-HPC and compact

this. After sieving of the compressed bodies, the granules formed are mixed with magnesium stearate and silicon dioxide and pressed on a tablet press to give tablets with a Ø of 9 mm.

- Moist granulation

e.g. per tablet:	25 mg	compound according to the invention
	205 mg	microcrystalline cellulose
	6 mg	povidone K30
	10 mg	crospovidone
	4 mg	magnesium stearate
	250 mg	total

Prepare a homogeneous mixture of the compound with the microcrystalline cellulose and the crospovidone and granulate this with an aqueous solution of the povidone in a granulator. The moist granules are then after-granulated and, after drying, dried in a drying cabinet (50°C) for 10 h. The dry granules are sieved together with the magnesium stearate, finally mixed and pressed on a tablet press to give tablets with a Ø of 8 mm.

Example 8:

Example of a medicament for pain treatment comprising a compound according to the invention - parenteral solution

1 g of a compound according to the invention is dissolved in 1 l water for injection purposes at room temperature and

the solution is then adjusted to isotonic conditions by addition of NaCl (sodium chloride).

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Patent claims

1. Method for detecting pain-regulating substances with the following process steps:

(a) incubation under suitable conditions of a substance to be tested with the protein BNPI or DNPI and/or a protein according to one of figures 1b), 1d), 1f), 2b) or 2d) and/or a protein which is similar to one of these abovementioned proteins to the extent of at least 90% and/or a protein for which a polynucleotide according to one of figures 1a), 1c), 1e), 2a) or 2c) or a polynucleotide which is similar thereto to the extent of at least 90% codes, and/or a protein which is coded by a nucleic acid which bind under stringent conditions to a polynucleotide according to one of figures 1a), 1c), 1e), 2a) or 2c) or antisense polynucleotides thereof, or a part protein of one of the abovementioned proteins which is at least 10, preferably at least 15, in particular at least 20 amino acids long and/or a cell and/or a preparation from such a cell which has synthesized at least one of the abovementioned proteins and part proteins,

(b) measurement of the binding of the test substance to the protein or part protein synthesized by the cell or measurement of at least one of the functional parameters modified by the binding of the test substance to the protein or part protein.

2. Method according to claim 1, characterized in that the cell is manipulated by genetic engineering before step (a).

3. Method according to claim 2, characterized in that the manipulation by genetic engineering allows the measurement of at least one functional parameter modified by the test substance.

4. Method according to claim 3, characterized in that, by the manipulation by genetic engineering, a form of a G protein which is not expressed endogenously in the cell is expressed or a reporter gene is introduced.

5. Method according to one of claims 2-4, characterized in that the cell is manipulated by genetic engineering such that the cell contains at least one polynucleotide according to one of figures 1a), 1c), 1e), 2a) or 2c) or a polynucleotide which is similar thereto to the extent of at least 90%.

6. Method according to claim 5, characterized in that the polynucleotide is contained in a recombinant DNA construct.

7. Method according to one of claims 2 to 6, characterized in that after the manipulation by genetic engineering according to claim 2 and before step (a) according to claim 1, the cell is cultured under conditions which allow an expression, optionally under selection pressure.

8. Method according to one of claims 1 to 7, characterized in that the cell is an amphibia cell, bacteria cell, yeast cell, insect cell or an immortalized or native mammalian cell.

9. Method according to one of claims 1 to 8, characterized in that the measurement of the binding is carried out via the displacement of a known labelled ligand of the part protein or protein and/or via the activity bound thereto from a labelled test substance.

10. Method according to one of claims 1 to 8, characterized in that the measurement of at least one of the functional parameters modified by the test substance is carried out via measurement of the regulation, inhibition and/or activation of receptors, ion channels and/or enzymes, in particular via measurement of the modification of the gene expression, the ionic medium, the pH or the membrane potential, via modification of the enzyme activity or the concentration of the 2nd messenger.

11. Method according to one of claims 1 to 10, characterized in that the pain regulated by the substance to be detected is chosen from:

chronic pain, in particular musculoskeletal pain; neuropathic pain, in particular allodynic pain, mechanical hyperalgesia or diabetic neuropathy; visceral pain, cerebral pain, peripheral pain or inflammation-related pain, in particular

peripheral inflammation pain; and migraine, cluster headache or pain with trigeminus neuralgia.

12. Compound identifiable as a pain-regulating substance by a method according to one of claims 1 to 11.

13. Compound according to claim 12, characterized in that it is a low molecular weight compound.

14. Use of

a. a polynucleotide, preferably a DNA or RNA, which codes for BNPI or DNPI or a polynucleotide, preferably a DNA or RNA, which corresponds to the extent of at least 90%, preferably 95%, in particular to the extent of at least 97%, to one of the nucleotide sequences shown in figures 1a), 1c), 1e), 2a), 2c) or 2e),

b. a polynucleotide, in particular an antisense polynucleotide or a PNA, preferably a DNA enzyme or ribozyme, a ribozyme or other DNA enzyme or a catalytic RNA or DNA, which has a nucleotide sequence which is capable of binding specifically to one of the polynucleotides listed under point a),

c. a vector containing a polynucleotide according to one of points a) or b), in particular an expression vector and/or in particular derived from a virus, for example the adenovirus, adeno-associated virus or herpes virus, and/or in

particular containing at least one LTR, poly A, promoter and/or ORI sequence,

d. BNPI or DNPI and/or a protein according to one of figures 1b), 1d), 1f), 2b) or 2d) and/or a protein which is similar to one of these abovementioned proteins to the extent of at least 90% and/or a protein for which a polynucleotide according to one of figures 1a), 1c), 1e), 2a) or 2c) or a polynucleotide which is similar thereto to the extent of at least 90% codes, and/or a protein which is coded by a nucleic acid which binds under stringent conditions to a polynucleotide according to one of figures 1a), 1c), 1e), 2a) or 2c) or antisense polynucleotides thereof or a part protein of one of the abovementioned proteins which is at least 10, preferably at least 15, in particular at least 20 amino acids long, wherein the protein or part protein has been optionally post-translationally modified, in particular glycosylated, phosphorylated, amidated, methylated, acetylated, ADP-ribosylated, hydroxylated, provided with a membrane anchor, cleaved or shortened,

e. an antibody, preferably a monoclonal or polyclonal antibody, against one of the proteins or part proteins according to point d),

f. a cell, preferably an amphibia cell, bacteria cell, yeast cell, insect cell or an immortalized or native mammalian cell, containing a polynucleotide according to one of points a) or b), a vector according to point c), a protein or

part protein according to point d) or an antibody according to point e)

g. a compound according to one of claims 12 or 13 and/or

h. an active compound, preferably a low molecular weight active compound, which binds to a protein or part protein according to point a),

for the preparation of a medicament for treatment of pain.

15. Use of

a. a polynucleotide, preferably a DNA or RNA, which codes for BNPI or DNPI or a polynucleotide, preferably a DNA or RNA, which corresponds to the extent of at least 90%, preferably 95%, in particular to the extent of at least 97%, to one of the nucleotide sequences shown in figures 1a), 1c), 1e), 2a), 2c) or 2e),

b. a polynucleotide, in particular an antisense polynucleotide or a PNA, preferably a DNA enzyme or ribozyme, a ribozyme or other DNA enzyme or a catalytic RNA or DNA, which has a nucleotide sequence which is capable of binding specifically to one of the polynucleotides listed under point a),

c. a vector containing a polynucleotide according to one of points a) or b), in particular an expression vector and/or in particular derived from a virus, for example the adenovirus, adeno-associated virus or herpes virus, and/or

in particular containing at least one LTR, poly A, promoter and/or ORI sequence,

f. a cell, preferably an amphibia cell, bacteria cell, yeast cell, insect cell or an immortalized or native mammalian cell, containing a polynucleotide according to one of points a) or b) or a vector according to point c)

for the preparation of a medicament for use in gene therapy.

16. Use according to claim 15, characterized in that the gene therapy is in vivo or in vitro gene therapy.

17. Use according to one of claims 15 or 16, characterized in that the medicament is a medicament for treatment of pain.

18. Use of

a. a polynucleotide, preferably a DNA or RNA, which codes for BNPI or DNPI or a polynucleotide, preferably a DNA or RNA, which corresponds to the extent of at least 90%, preferably 95%, in particular to the extent of at least 97%, to one of the nucleotide sequences shown in figures 1a), 1c), 1e), 2a), 2c) or 2e),

b. a polynucleotide, in particular an antisense polynucleotide or a PNA, preferably a DNA enzyme or ribozyme, a ribozyme or other DNA enzyme or a catalytic RNA or DNA, which has a nucleotide sequence which is capable of binding

specifically to one of the polynucleotides listed under point a),

c. a vector containing a polynucleotide according to one of points a) or b), in particular an expression vector and/or in particular derived from a virus, for example the adenovirus, adeno-associated virus or herpes virus, and/or in particular containing at least one LTR, poly A, promoter and/or ORI sequence,

d. BNPI or DNPI and/or a protein according to one of figures 1b), 1d), 1f), 2b) or 2d) and/or a protein which is similar to one of these abovementioned proteins to the extent of at least 90% and/or a protein for which a polynucleotide according to one of figures 1a), 1c), 1e), 2a) or 2c) or a polynucleotide which is similar thereto to the extent of at least 90% codes, and/or a protein which is coded by a nucleic acid which binds under stringent conditions to a polynucleotide according to one of figures 1a), 1c), 1e), 2a) or 2c) or antisense polynucleotides thereof or a part protein of one of the abovementioned proteins which is at least 10, preferably at least 15, in particular at least 20 amino acids long, wherein the protein or part protein has been optionally post-translationally modified, in particular glycosylated, phosphorylated, amidated, methylated, acetylated, ADP-ribosylated, hydroxylated, provided with a membrane anchor, cleaved or shortened,

e. an antibody, preferably a monoclonal or polyclonal antibody, against one of the proteins or part proteins according to point d),

f. a cell, preferably an amphibia cell, bacteria cell, yeast cell, insect cell or an immortalized or native mammalian cell, containing a polynucleotide according to one of points a) or b), a vector according to point c), a protein or part protein according to point d) or an antibody according to point e)

g. a compound according to one of claims 12 or 13 and/or

h. an active compound, preferably a low molecular weight active compound, which binds to a protein or part protein according to point a),

for the preparation of a diagnostic agent for diagnosis of a pain state.

19. Use of

a. a polynucleotide, preferably a DNA or RNA, which codes for BNPI or DNPI or a polynucleotide, preferably a DNA or RNA, which corresponds to the extent of at least 90%, preferably 95%, in particular to the extent of at least 97%, to one of the nucleotide sequences shown in figures 1a), 1c), 1e), 2a), 2c) or 2e),

b. a polynucleotide, in particular an antisense polynucleotide or a PNA, preferably a DNA enzyme or ribozyme, a ribozyme or other DNA enzyme or a catalytic RNA or DNA, which has a nucleotide sequence

which is capable of binding specifically to one of the polynucleotides listed under point a),

c. a vector containing a polynucleotide according to one of points a) or b), in particular an expression vector and/or in particular derived from a virus, for example the adenovirus, adeno-associated virus or herpes virus, and/or in particular containing at least one LTR, poly A, promoter and/or ORI sequence,

d. BNPI or DNPI and/or a protein according to one of figures 1b), 1d), 1f), 2b) or 2d) and/or a protein which is similar to one of these abovementioned proteins to the extent of at least 90% and/or a protein for which a polynucleotide according to one of figures 1a), 1c), 1e), 2a) or 2c) or a polynucleotide which is similar thereto to the extent of at least 90% codes, and/or a protein which is coded by a nucleic acid which binds under stringent conditions to a polynucleotide according to one of figures 1a), 1c), 1e), 2a) or 2c) or antisense polynucleotides thereof or a part protein of one of the abovementioned proteins which is at least 10, preferably at least 15, in particular at least 20 amino acids long, wherein the protein or part protein has been optionally post-translationally modified, in particular glycosylated, phosphorylated, amidated, methylated, acetylated, ADP-ribosylated, hydroxylated, provided with a membrane anchor, cleaved or shortened,

e. an antibody, preferably a monoclonal or polyclonal antibody, against one of the proteins or part proteins according to point d),

f. a cell, preferably an amphibia cell, bacteria cell, yeast cell, insect cell or an immortalized or native mammalian cell, containing a polynucleotide according to one of points a) or b), a vector according to point c), a protein or part protein according to point d) or an antibody according to point e)

in a method for detecting pain-regulating substances.

20. Use according to one of claims 14, 17, 18 or 19, characterized in that the pain is chosen from

chronic pain, in particular musculoskeletal pain; neuropathic pain, in particular allodynic pain, mechanical hyperalgesia or diabetic neuropathy; visceral pain, cerebral pain, peripheral pain or inflammation-related pain, in particular peripheral inflammation pain; and migraine, cluster headache or pain with trigeminus neuralgia.

Abstract

The invention relates to a method for detecting pain-regulating substances using BNPI and/or DNPI and the use of compounds thereby identified, active compounds which bind to BNPI and/or DNPI, antibodies directed against BNPI and/or DNPI, of antisense nucleotides against BNPI and/or DNPI, or of BNPI and/or DNPI or part proteins thereof, and corresponding polynucleotides for medicaments for pain therapy and diagnostic agents.

Fig. 1a)

```
ccggcggcag gagccgccac catggagttc cgccaggagg agtttcggaa gctagcgggt 60
cgtgctctcg ggaagctgca ccgccttctg gagaagcggc aggaaggcgc ggagacgctg 120
gagctgagtg cggatgggcg cccggtgacc acgcagaccc gggacccgcc ggtggtggac 180
tgcacctgct tcggcctccc tcgccgctac attatcgcca tcatgagtgg tctgggcttc 240
tgcatcagct ttggcatccg ctgcaacctg ggcgtggcca tcgtctccat ggtcaataac 300
agcacgaccc accgcggggg ccacgtggtg gtgcagaaag cccagttcag ctgggatcca 360
gagactgtcg gcctcataca cggctccttt ttctggggct acattgtcac tcagattcca 420
ggaggattta tctgtcaaaa atttgcagcc aacagagttt tcggctttgc tattgtggca 480
acatccactc taaacatgct gatcccctca gctgcccgcg tccactatgg ctgtgtcatc 540
ttcgtgagga tcctgcaggg gttggtagag ggggtcacat accccgcctg ccatgggatc 600
tggagcaaat gggccccacc cttagaacgg agtcgcctgg cgacgacagc cttttgtggt 660
tcctatgctg gggcggtggt cgcgatgccc ctcgccgggg tccttgtgca gtactcagga 720
tggagctctg ttttctacgt ctacggcagc ttcgggatct tctggtacct gttctggctg 780
ctcgtctcct acgagtcccc cgcgctgcac cccagcatct cggaggagga gcgcaagtac 840
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ccctggcggc gcttcttcac gtctatgcca gtctatgcca tcatcgtggc caacttctgc 960
cgcagctgga cgttctacct gctgctcatc tcccagcccg cctacttcga agaagtgttc 1020
ggcttcgaga tcagcaaggt aggcctggtg tccgcgctgc cccacctggt catgaccatc 1080
atcgtgccca tcggcggcca gatcgcggac ttcctgcgga gccgccgcat catgtccacc 1140
accaacgtgc gcaagttgat gaactgcgga ggcttcggca tggaagccac gctgctgttg 1200
gtggtcggct actcgcactc caagggcgtg gccatctcct tcctggtcct agccgtgggc 1260
ttcagcggct tcgccatctc tgggttcaac gtgaaccacc tggacatagc cccgcgctac 1320
gccagcatcc tcatgggcat ctccaacggc gtgggcacac tgtcgggcat ggtgtgcccc 1380
atcatcgtgg gggccatgac taagcacaag actcgggagg agtggcagta cgtgttccta 1440
attgcctccc tggtgcacta tggaggtgtc atcttctacg gggtctttgc ttctggagag 1500
aagcagccgt gggcagagcc tgaggagatg agcgaggaga agtgtggctt cgttggccat 1560
gaccagctgg ctggcagtga cgacagcgaa atggaggatg aggctgagcc cccggggcca 1620
ccccctgcac ccccgccctc ctatggggcc acacacagca catttcagcc ccccaggccc 1680
ccacccccctg tccgggacta ctgaccatgt gcctcccact gaatggcagt ttccaggacc 1740
tccattccac tcatctctgg cctgagtgac agtgtcaagg aaccctgctc ctctctgtcc 1800
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ctcaccagcg tttctgatgg aaaatggcgg tttcaagtcc ccgattccgt gcccacttca 2280
catctccccc accagcagat tctgcgaaag caccaaattt ctcaagaccc tcttctccct 2340
agcttagcat aatgtctggg gaaaca
```

Fig. 1b)

```
1    MEFRQEEFRK LAGRALGKLH RLLEKRQEGA ETLELSADGR PVTTQTRDPP
51   VVDCTCFGLP RRYIIAIMSG LGFCISFGIR CNLGVAIVSM VNNSTTHRGG
101  HVVVQKAQFS WDPETVGLIH GSFFWGYIVT QIPGGFICQK FAANRVFGFA
151  IVATSTLNML IPSAARVHYG CVIFVRILQG LVEGVTYPAC HGIWSKWAPP
201  LERSRLATTA FCGSYAGAVV AMPLAGVLVQ YSGWSSVFYV YGSFGIFWYL
251  FWLLVSYESP ALHPSISEEE RKYIEDAIGE SAKLMNPLTK FSTPWRRFFT
301  SMPVYAIIVA NFCRSWTFYL LLISQPAYFE EVFGFEISKV GLVSALPHLV
351  MTIIVPIGGQ IADFLRSRRI MSTTNVRKLM NCGGFGMEAT LLLVVGYSHS
401  KGVAISFLVL AVGFSGFAIS GFNVNHLDIA PRYASILMGI SNGVGTLSGM
451  VCPIIVGAMT KHKTREEWQY VFLIASLVHY GGVIFYGVFA SGEKQPWAEP
501  EEMSEEKCGF VGHDQLAGSD DSEMEDEAEP PGAPPAPPPS YGATHSTFQP
551  PRPPPPVRDY
```

Fig. 1c)

```
cgataagctt gatatcgaat tccggactct tgctcgggcg ccttaacccg gcgttcggtt 60
catcccgcag cgccagttct gcttaccaaa agtggcccac taggcactcg cattccacgc 120
ccggctccac gccagcgagc cgggcttctt acccatttaa agtttgagaa taggttgaga 180
tcgtttcggc cccaagacct ctaatcattc gctttaccgg ataaaactgc gtggcggggg 240
tgcgtcgggt ctgcgagagc gccagctatc ctgagggaaa cttcggaggg aaccagctac 300
tagatggttc gattagtctt tcgcccctat acccaggtcg gacgaccgat ttgcacgtca 360
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gggggggaccc gcggggtgac cggcggcagg agccgccacc atggagttcc gccaggagga 480
gtttcggaag ctagcgggtc gtgctctcgg gaagctgcac cgccttctgg agaagcggca 540
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cgtctccatg gtcaataaca gcacgaccca ccgcgggggc cacgtggtgg tgcagaaagc 780
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ccacctggtc atgaccatca tcgtgcccat cggcggccag atcgcggact tcctgcggag 1560
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gccattccag ggattgattc tcaccagcgt ttctgatgga aaatggcggg aattcctgca 2700
gcccgggggga tccact                                              2716
```

Fig. 1d)

```
1    MEFRQEEFRK LAGRALGKLH RLLEKRQEGA ETLELSADGR PVTTQTRDPP
51   VVDCTCFGLP RRYIIAIMSG LGFCISFGIR CNLGVAIVSM VNNSTTHRGG
101  HVVVQKAQFS WDPETVGLIH GSFFWGYIVT QIPGGFICQK FAANRVFGFA
151  IVATSTLNML IPSAARVHYG CVIFVRILQG LVEGVTYPAC HGIWSKWAPP
201  LERSRLATTA FCGSYAGAVV AMPLAGVLVQ YSGWSSVFYV YGSFGIFWYL
251  FWLLVSYESP ALHPSISEEE RKYIEDAIGE SAKLMNPLTK FSTPWRRFFT
301  SMPVYAIIVA NFCRSWTFYL LLISQPDYFE EVFGFEISKV GLVSALPHLV
351  MTIIVPIGGQ IADFLRSRRI MSTTNVRKLM NCGGFGMEAT LLLVVGYSHS
401  KGVAISFLVL AVGFSGFAIS GFNVNHLDIA PRYASILMGI SNGVGTLSGM
451  VCPIIVGAMT KHKTREEWQY VFLIASLVHY GGVIFYGVFA SGEKQPWAEP
501  EEMSEEKCGF VGHDQLAGSD DSEMEDEAEP PGAPPAPPPS YGATHSTFQP
551  PRPPPPVRDY
```

Fig. 1e)

```
gaattcggca cgagcggagc tgcggggccg ggccgggccg gggcggaccc cgggatcccg 60
gacgcggccg cccgggcccg cgggcggggg gattggcagg ggacccgcgt gggcacagcc 120
accatggagt tccggcagga ggagtttcgg aagctggcgg ggcgcgccct ggggaggctg 180
caccggttac tggagaagcg gcaggaaggc gcggagacat tggagctgag cgccgacggg 240
cgcccagtga ccacacacac gcgggacccg ccggtggtgg actgcacttg ctttggcctc 300
cctcgccgct acatcatcgc gatcatgagc ggtctgggtt tctgcatcag ctttggcatc 360
cgctgcaacc tgggcgtggc catcgtatcc atggtcaaca acagtacaac ccaccgtggg 420
ggccacgtgg tggtgcagaa agcccagttc aactgggatc cagagactgt cggcctcata 480
catggctcct ttttctgggg gtacattgtc actcagattc ctggaggatt tatctgccaa 540
aaattcgcag ccaacagggt ctttggcttt gccattgtgg ctacctccac cctaaatatg 600
ttgatccctt cagcagcccg tgttcactat ggctgtgtca tcttcgtgag gatccttcag 660
ggattggtgg agggggtcac ataccctgct tgccatggca tctggagcaa atgggcccct 720
cccttagaac ggagtcggct ggcgacgaca gcctttgcg gttcctatgc cggggcagtg 780
gttgccatgc ctctggctgg ggtcctggta cagtattcag gatggagttc tgtcttctat 840
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cctgcactac accccagcat ctccgaggag gagcgcaaat acattgagga tgccatcgga 960
gaaagcgcca agctcatgaa ccctgttacg aagtttaaca caccctggag gcgcttcttt 1020
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cagatcgccg acttcctgcg cagtcgtcat ataatgtcca cgaccaatgt gcgaaagctg 1260
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tccaagggcg tggccatctc cttcctggtc ctggctgtgg gcttcagtgg ctttgctatc 1380
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ccggaggaga tgagcgagga gaagtgtggc tttgttggcc acgaccagct ggctggcagt 1680
gacgaaagtg aaatggaaga cgaggttgag ccccggggg caccccccgc acctccgcct 1740
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tcttcccttg tgcccagtgc tgtccgacca gccctctctc cttctcaact gcctcttgca 1980
ggggtgaagc tgcacactag cagtttcaag ctcgtgccga attc
```

Fig. 1f)

```
1     MEFRQEEFRK LAGRALGRLH RLLEKRQEGA ETLELSADGR PVTTHTRDPP VVDCTCFGLP
51    RRYIIAIMSG LGFCISFGIR CNLGVAIVSM VNNSTTHRGG HVVVQKAQFN WDPETVGLIH
101   GSFFWGYIVT QIPGGFICQK FAANRVFGFA IVATSTLNML IPSAARVHYG CVIFVRILQG
151   LVEGVTYPAC HGIWSKWAPP LERSRLATTA FCGSYAGAVV AMPLAGVLVQ YSGWSSVFYV
201   YGSFGIFWYL FWLLVSYESP ALHPSISEEE RKYIEDAIGE SAKLMNPVTK FNTPWRRFFT
251   SMPVYAIIVA NFCRSWTFYL LLISQPAYFE EVFGFEISKV GLVSALPHLV MTIIVPIGGQ
301   IADFLRSRHI MSTTNVRKLM NCGGFGMEAT LLLVVGYSHS KGVAISFLVL AVGFSGFAIS
351   GFNVNHLDIA PRYASILMGI SNGVGTLSGM VCPIIVGAMT KHKTREEWQY VFLIASLVHY
401   GGVIFYGVFA SGEKQPWAEP EEMSEEKCGF VGHDQLAGSD ESEMEDEVEP PGAPPAPPPS
451   YGATHSTVQP PRPPPPVRDY
```

Fig. 2a)

```
cgtttaaaag ccatcagatt tgagagcaat aagtcttcaa aaccgggaat ttacattgtt 60
tttcagctga ccgacttcca ggaaaaggac tcaaccgcat ctacccaaat accgtggcac 120
tgcttgcgct ctttgccacc ggatactccc cttccaatga gactttctga ttgtgtctac 180
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ccgtaaaaca aaggattttg gccccaggaa aagaggggct aaagaatttt gctggaaaat 480
cactcggcca gatctacagg gtgctggaga agaagcaaga caccggggag acaatcgagc 540
tgacggagga tgggaagccc ctagaggtgc ccgagaggaa ggcgccgctg tgcgactgca 600
cgtgcttcgg cctgccccgc cgctacatta tcgccatcat gagcggcctg ggcttctgca 660
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ccatccaccg cgggggcaag gtcatcaagg agaaagccaa attcaactgg gacccggaaa 780
ccgtggggat gatccacggt tccttctttt ggggctacat catcactcag attccgggag 840
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cttcagtgtt ttatgtctac ggaagctttg gaatggtctg gtacatgttt tggcttttgg 1200
tgtcttatga aagtcctgca aagcatccta ctattacaga tgaagaacgt aggtacatag 1260
aagaaagcat tggagagagt gcaaatcttt taggtgcaat ggaaaaattc aagactccat 1320
ggaggaagtt ttttacatcc atgccagtct atgcaataat tgttgcaaac ttctgcagaa 1380
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tgcctattgg gggacaaatt gcagattttc taagaagcaa gcagattctt tcaactacga 1560
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ttgttggtgc aatgacaaag aataagtcac gtgaagagtg gcagtatgtc ttcctgatcg 1860
ctgccctagt ccactatggt ggagttatat tttatgcaat atttgcctca ggagagaaac 1920
aaccctgggc agacccggag gaaacaagtg aagaaaaatg tggatttatt catgaagatg 1980
aactcgatga agaaacaggg gacattactc aaaattatat aaattatggt accaccaagt 2040
cttatggtgc cacaacacag gccaatggag gttggcctag tggttgggaa aagaaagagg 2100
aatttgtaca aggagaagta caagactcac atagctataa ggaccgagtt gattattcat 2160
aacaaaacta attactggat ttatttttag tgtttgtgat taaattcatt gtgattgcac 2220
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Fig. 2 b)

```
1     MESVKQRILA PGKEGLKNFA GKSLGQIYRV LEKKQDTGET IELTEDGKPL EVPERKAPLC
61    DCTCFGLPRR YIIAIMSGLG FCISFGIRCN LGVAIVDMVN NSTIHRGGKV IKEKAKFNWD
121   PETVGMIHGS FFWGYIITQI PGGYIASRLA ANRVFGAAIL LTSTLNMLIP SAARVHYGCV
181   IFVRILQGLV EGVTYPACHG IWSKWAPPLE RSRLATTSFC GSYAGAVIAM PLAGILVQYT
241   GWSSVFYVYG SFGMVWYMFW LLVSYESPAK HPTITDEERR YIEESIGESA NLLGAMEKFK
301   TPWRKFFTSM PVYAIIVANF CRSWTFYLLL ISQPAYFEEV FGFEISKVGM LSAVPHLVMT
361   IIVPIGGQIA DFLRSKQILS TTTVRKIMNC GGFGMEATLL LVVGYSHTRG VAISFLVLAV
421   GFSGFAISGF NVNHLDIAPR YASILMGISN GVGTLSGMVC PIIVGAMTKN KSREEWQYVF
481   LIAALVHYGG VIFYAIFASG EKQPWADPEE TSEEKCGFIH EDELDEETGD ITQNYINYGT
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```

Fig. 2c)

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Fig. 2d)

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101  NSTIHRGGKV IKEKAKFNWD PETVGMIHGS FFWGYIITQI PGGYIASRLA
151  ANRVFGAAIL LTSTLNMLIP SAARVHYGCV IFVRILQGLV EGVTYPACHG
201  IWSKWAPPLE RSRLATTSFC GSYAGAVIAM PLAGILVQYT GWSSVFYVYG
251  SFGMVWYMFW LLVSYESPAK HPTITDEERR YIEESIGESA NLLGAMEKFK
301  TPWRKFFTSM PVYAIIVANF CRSWTFYLLL ISQPAYFEEV FGFEISKVGM
351  LSAVPHLVMT IIVPIGGQIA DFLRSKQILS TTTVRKIMNC GGFGMEATLL
401  LVVGYSHTRG VAISFLVLAV GFSGFAISGF NVNHLDIAPR YASILMGISN
451  GVGTLSGMVC PIIVGAMTKN KSREEWQYVF LIAALVHYGG VIFYALFASG
501  EKQPWADPEE TSEEKCGFIH EDELDEETGD ITQNYINYGT TKSYGATSQE
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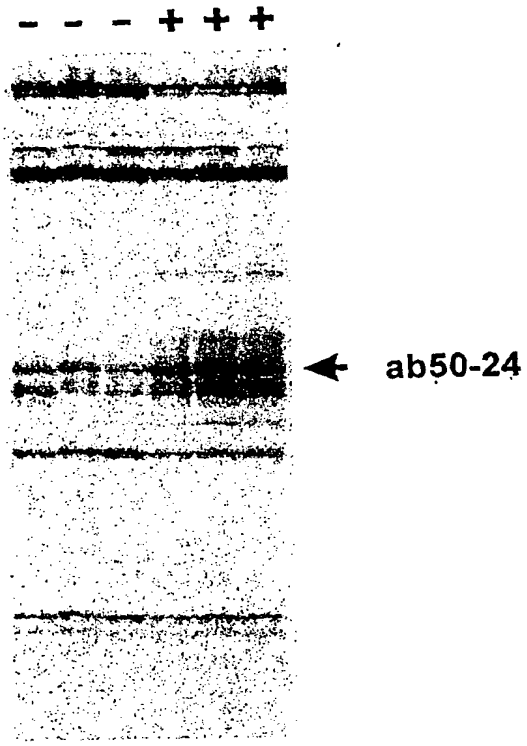
FIG 3)



7

FIG. 4)

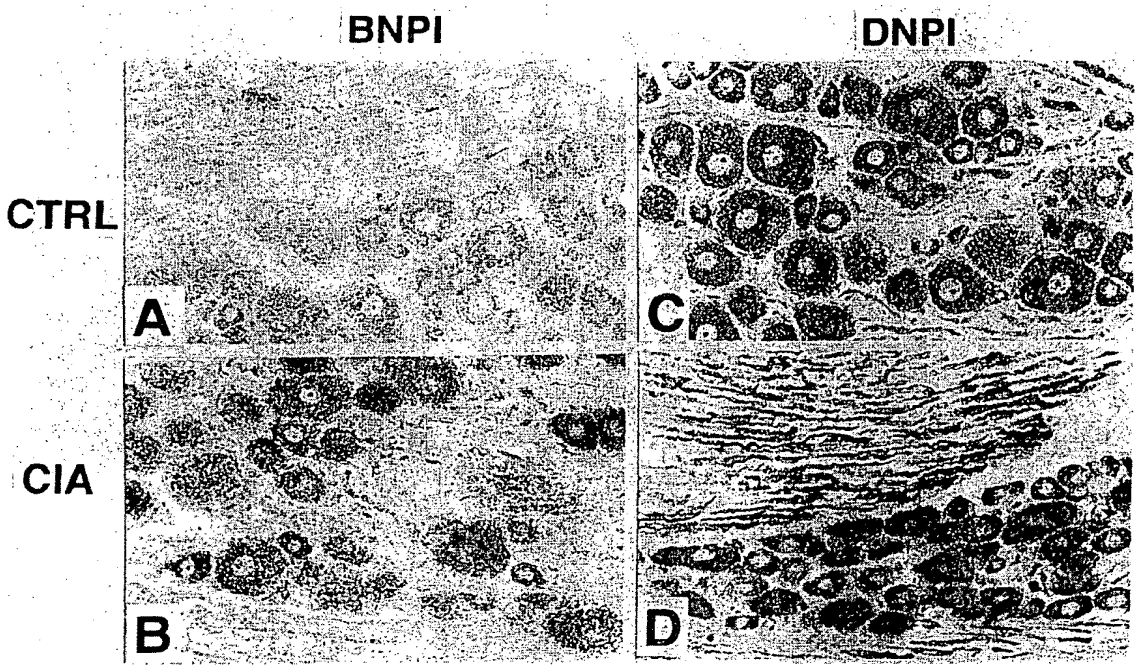


FIG 5)

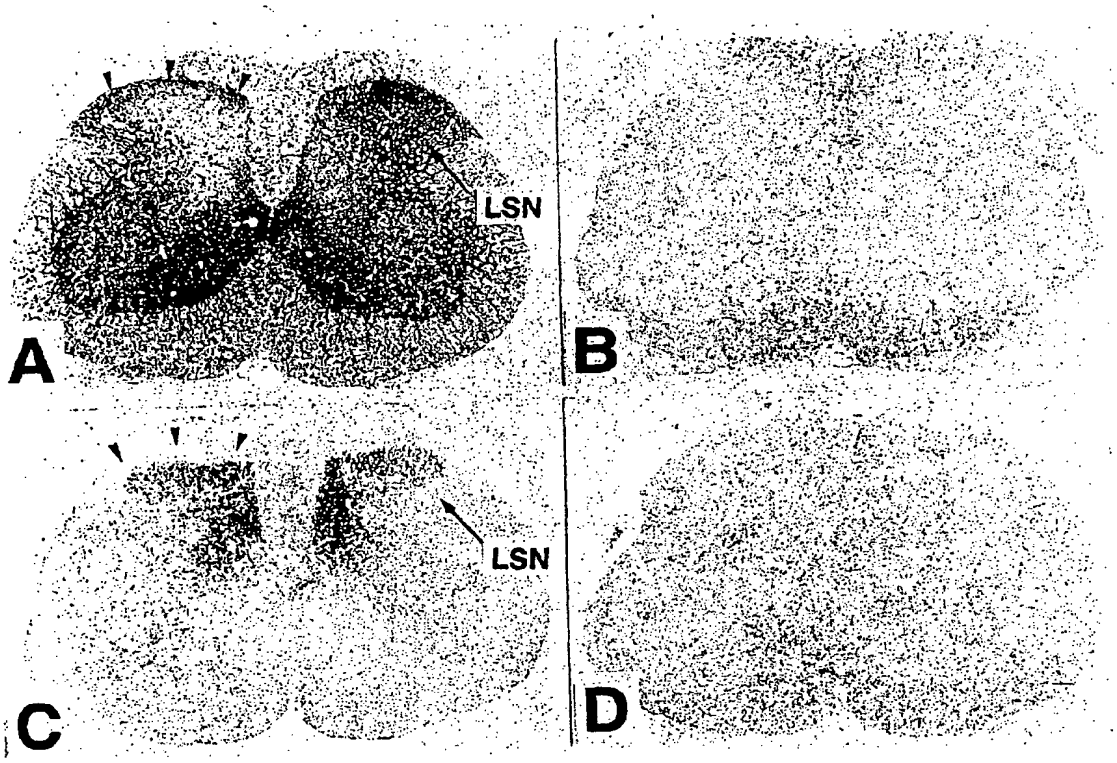
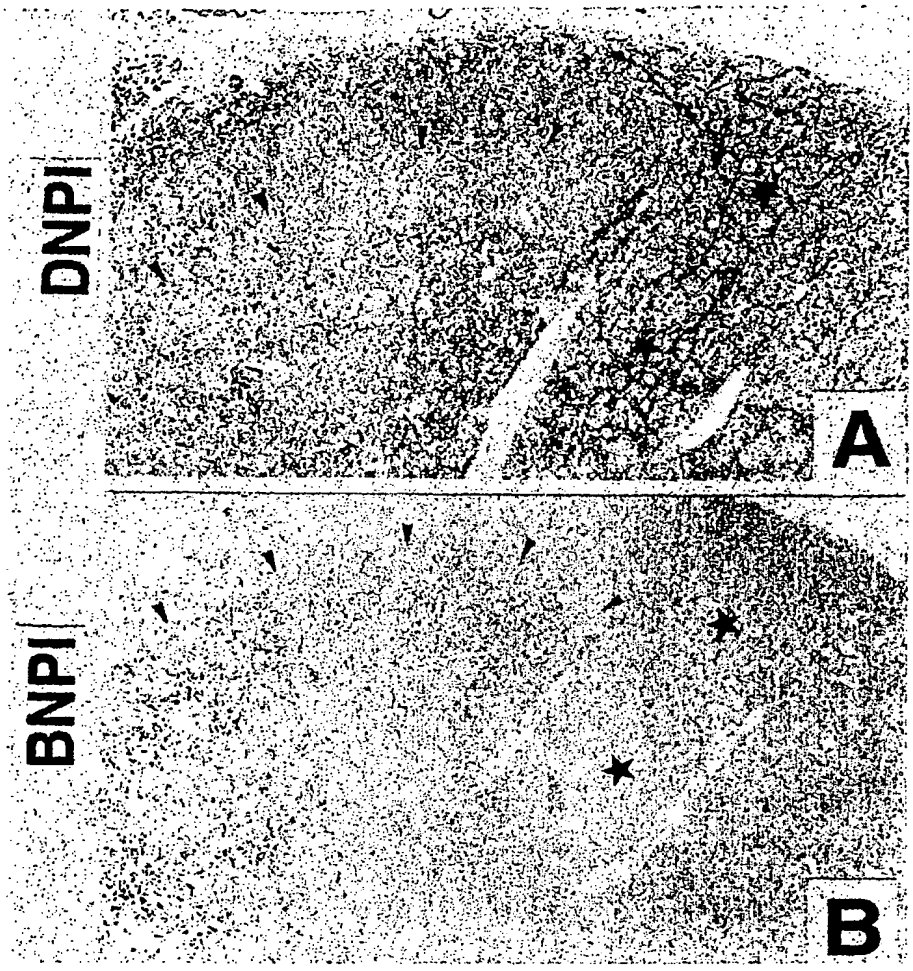


FIG 6)



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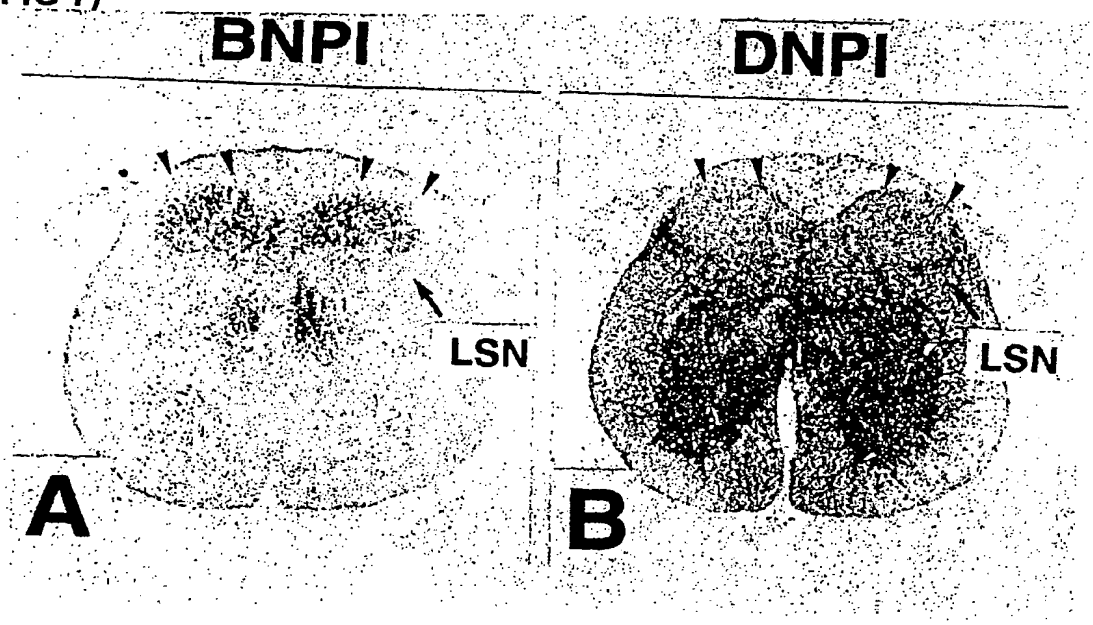

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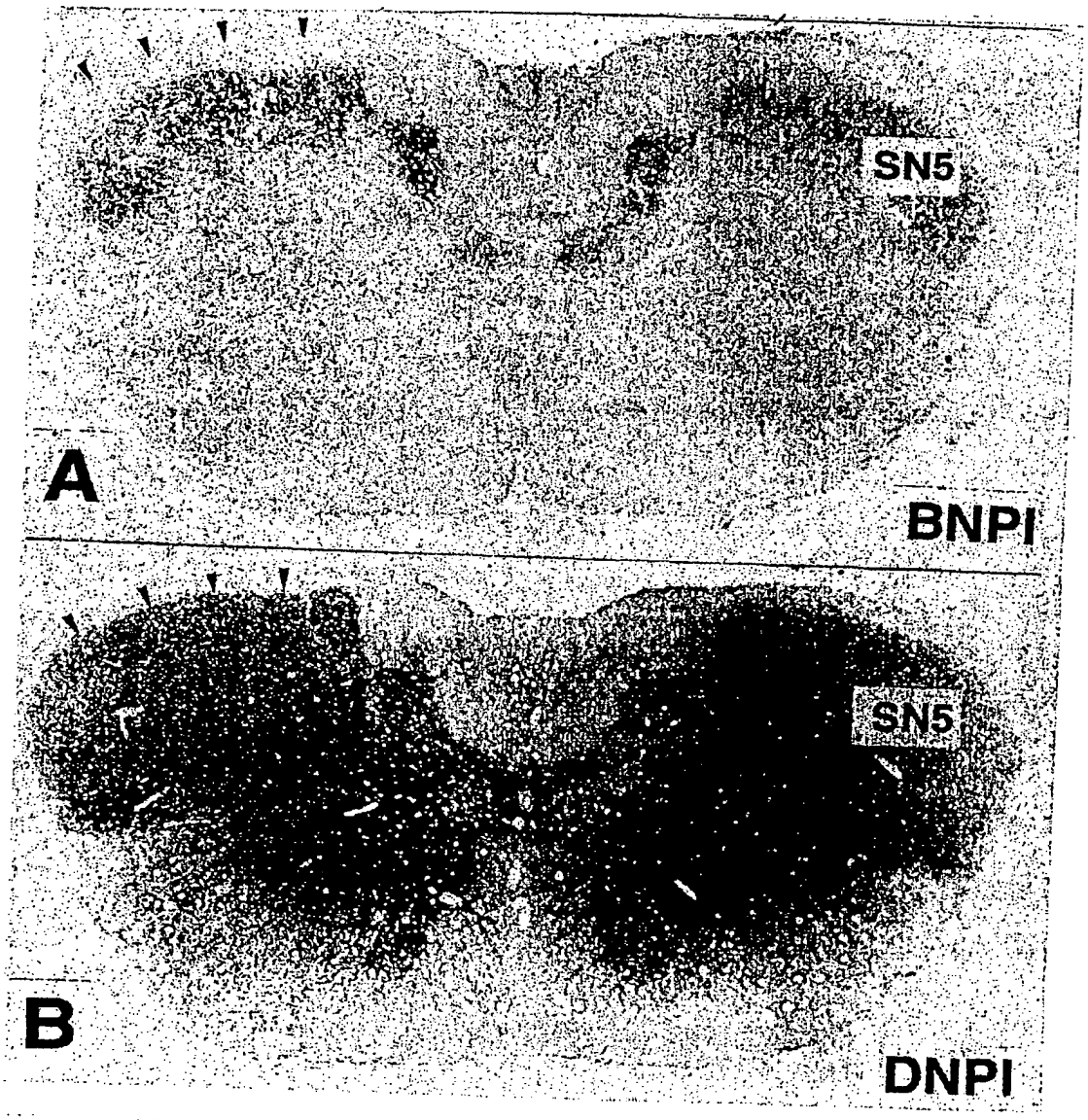

FIG 9)

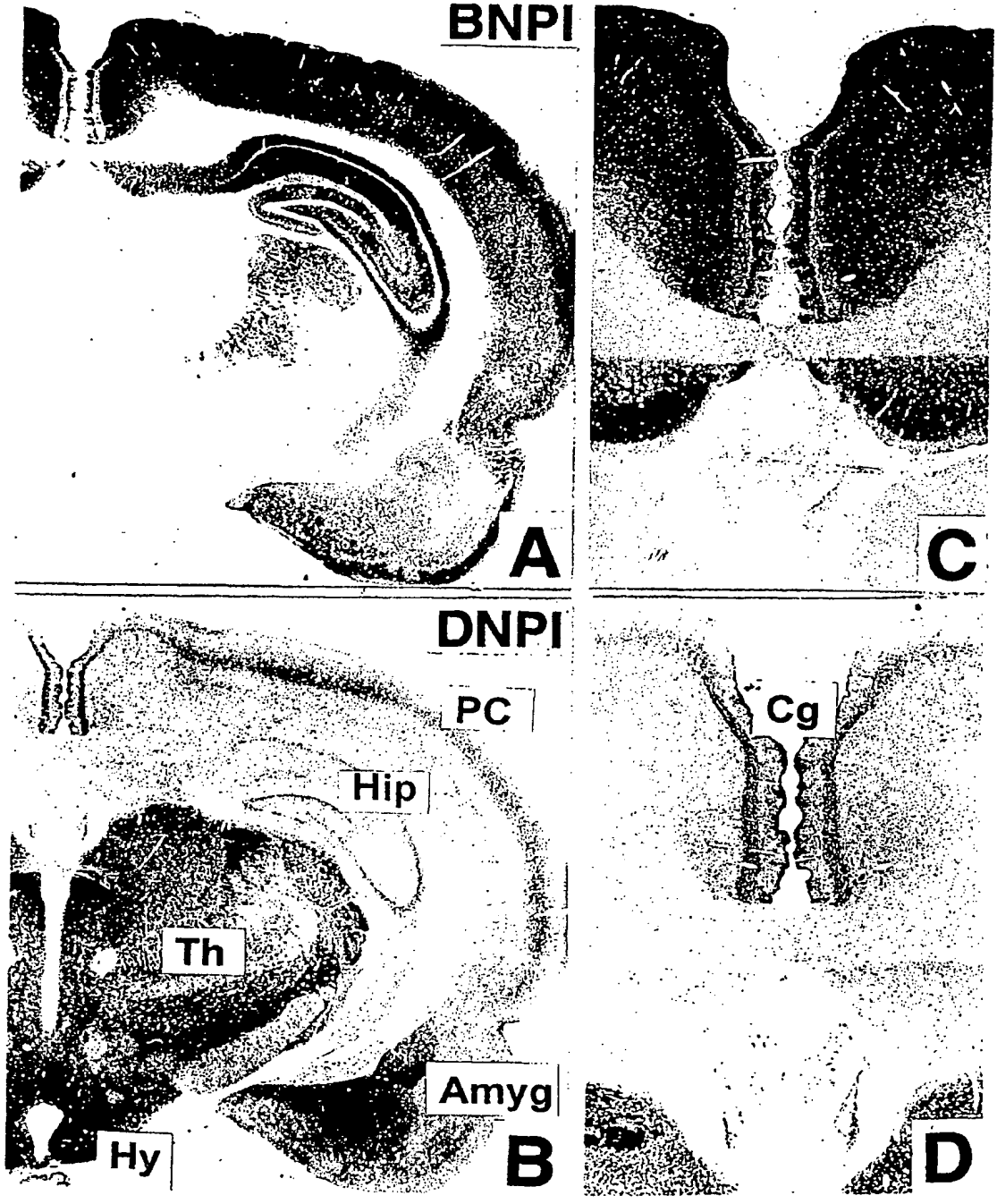

FIG 10)

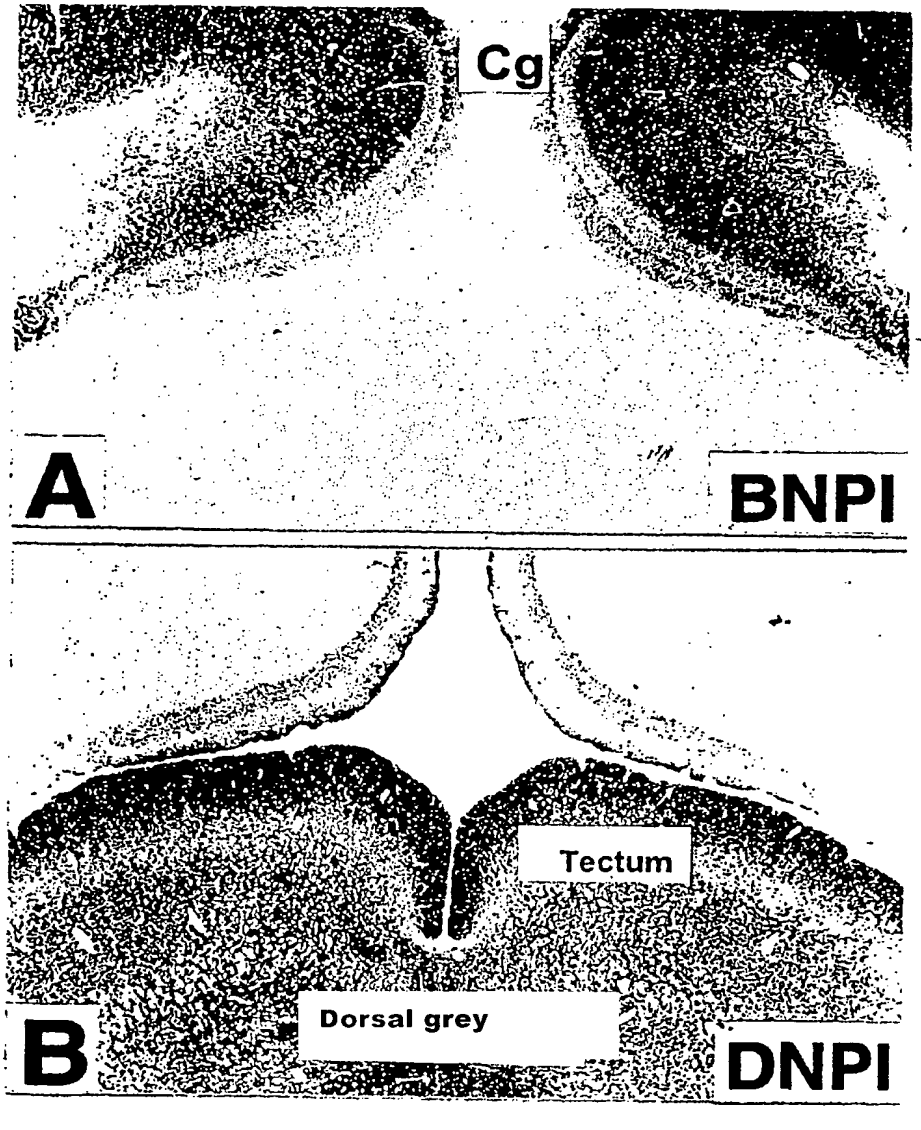

FIG 11)

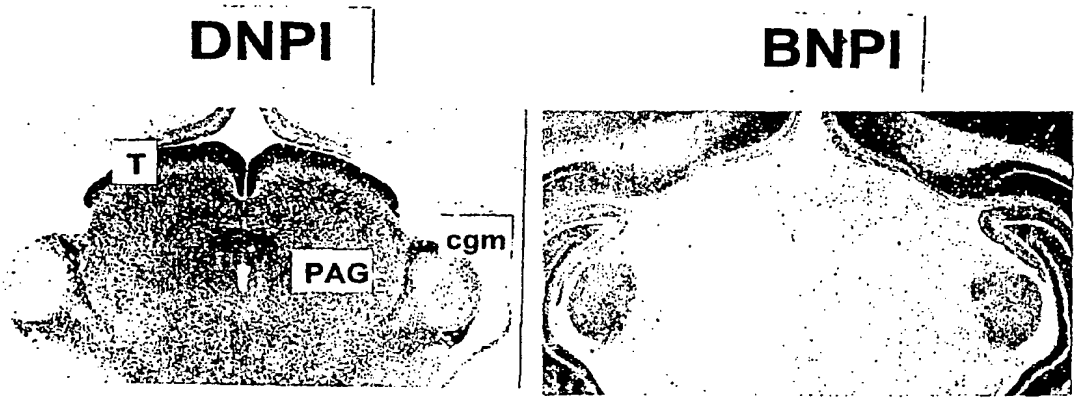

FIG 12)